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Circadian Rhythms - Genetic Regulation and Clinical Disorders

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INTRODUCTION

Circadian rhythms are endogenously generated rhythms with a period length of about 24-hours. A biological clock in the hypothalamic suprachiasmatic nuclei is responsible for the generation of circadian rhythms. Notable examples of the circadian rhythms include the sleep-wake cycle and rhythms in hormone production. Abnormalities of the circadian system include biological clock lesions that result in arrhythmic behavior and irregular sleep patterns. Abnormalities of the circadian system also occur when there is desynchronization of environmental clock time with the phase of the "internal milieu" resulting in conditions such as "jet lag". Numerous aspects of human physiology are greatly influenced by the time of day, as is the pathogenesis of illness.

This review summarizes our current knowledge of the organization of the circadian system and the generation and regulation of biological clock function. The role the circadian system plays in human physiology along with the detection and treatment of biological clock disorders is also discussed.

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Robert M. Blizzard, MD
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CIRCADIAN SYSTEM ORGANIZATION

The system responsible for the generation and regulation of circadian rhythms is the circadian timing system. This neural system consists of a biological clock located in the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, of an input pathway from the retina, and output pathways from SCN (Figure 1).[1]

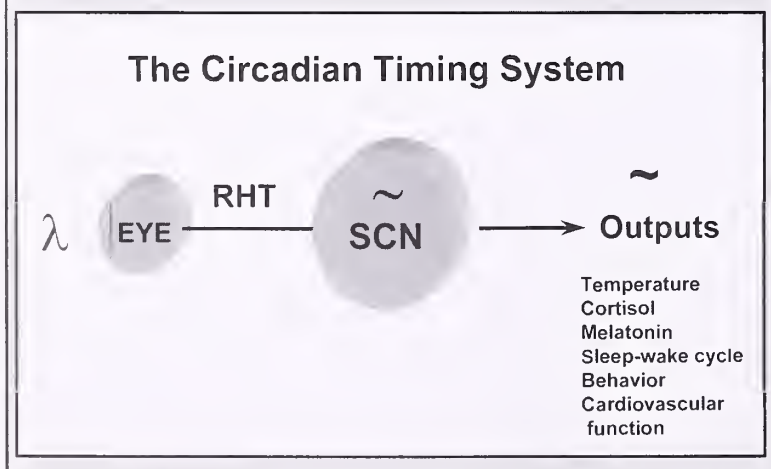
Because oscillations of the biological clock are not exactly 24-hours, synchronizing (entraining) the circadian pacemaker each day to the 24-hour light-dark cycle is necessary. Otherwise, clock oscillations will drift (free-run) out of phase with that of the environmental cycle. A direct pathway, the retinohypothalamic tract (RHT), from the retina to the SCN mediates photic entrainment of the SCN.[1] Light is the most potent entraining stimulus (Figure 1).

Two types of photic regulation of circadian phase (types 0 and 1) have been described.[2] In humans, strong (type 0) resetting is observed after very bright light exposure (10,000 lux), and modest (type 1) resetting is observed with ordinary indoor lighting (200 lux). Although cutaneous light has been suggested as influencing circadian function in humans, there is little support for the notion that this or other extraretinal photoreception is important in mammals.[3]

MOLECULAR BASIS OF CIRCADIAN RHYTHMICITY

Recent data suggests that the SCN is composed of multiple, single cell circadian oscillators. These oscillate as an ensemble to generate overt rhythms.[4] Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, plays an important role in synchronizing the oscillations of individual clock cells.[4]

Figure 1



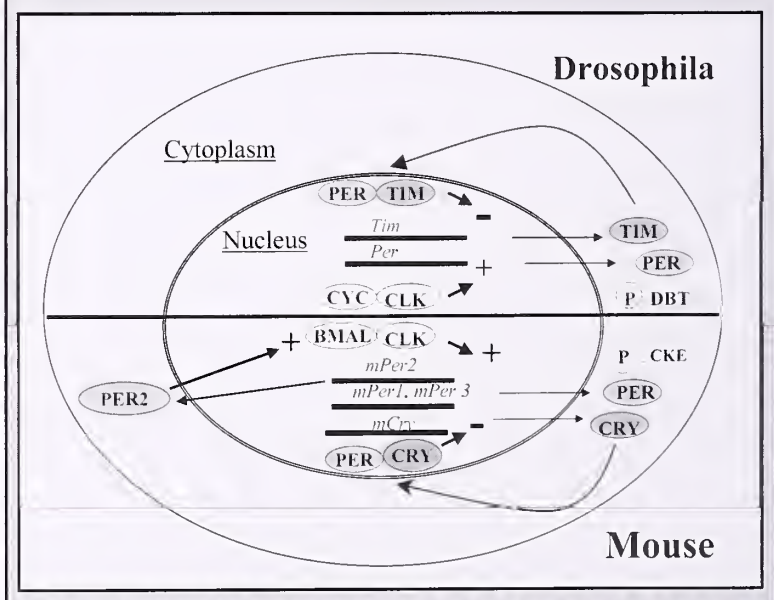
Schematic representation of the circadian timing system. RHT, retinohypothalamic tract; SCN, suprachiasmatic nuclei; λ, light. Modified from Rivkees SA, Hao H.

Considerable progress has been made over the past several years in defining the molecular mechanisms of clock oscillations.[5] In yeast, drosophila, and in mammals, it now appears that the molecular clockwork involves interlocking feedback loops that stimulate or inhibit clock gene expression.[6]

The molecular mechanisms leading to circadian rhythm generation were first detailed in drosophila (Figure 2). In these flies, the circadian feedback loop is generated by the transcriptional regulatory proteins PERIOD (PER) and TIMELESS (TIM) encoded by the *per* and *tim* genes. These are activated in the morning, and their two protein products accumulate in the cytoplasm during the day. In the evening, dimerization of PER and TIM occurs and the complex enters the nucleus. After entering the nuclei, the PER-TIM complex inhibits *per* and *tim* gene expression. In addition to feedback inhibition, the proteins CYCLE (CYC) and CLOCK (CLK) dimerize to stimulate *per* and *tim* gene expression in a rhythmic manner. These processes result in a 24-hour cycle of clock protein oscillations.

In the mammalian clock, several clock genes that are homologous to drosophila clock genes have been recently identified and discovered to play similar roles in clock regulation. Homologous mammalian and

Figure 2



Model of circadian clockwork in Drosophila and mouse. **In drosophila (top)**, CLOCK:CYCLE (CLK;CYC) heterodimers activate (+) rhythmic transcription of *Per* and *Tim*. The PERIOD (PER) and TIMELESS (TIM) proteins form complexes that are important for nuclear translocation. The PER:TIM complex, in turn inhibits (-) *Per* and *Tim* gene expression. The phosphorylation state of PER involves double-time (DBT). **In mouse (bottom)**, CLOCK:BMAL1 heterodimers activate (+) rhythmic transcription of *mPer1-3* and *mCry* genes. PER and CRYPTOCHROME (CRY) proteins form complexes important for nuclear translocation. The PER:CRY complex, in turn, inhibits *mPer* and *mCry* gene expression. The phosphorylation (P) state of the PER proteins involves casein kinase 1 epsilon (CKE). PER2 also stimulates *mBmal1* gene transcription.

Drosophila clock genes are described in Table 1, and their corresponding roles in circadian rhythm generation are illustrated in Figure 2. The rhythmic transcription of *mPer* genes (murine Pers 1-3) and *mCry* (Cryptochromes 1 and 2) are driven by the transcriptional activating factors CLOCK and BMAL1, that interact with specific promoter elements. PER and CRY then accumulate in the cytoplasm to form complexes that enter the nucleus. Within the nucleus, CRY will then directly interact with CLOCK and BMAL1 to turn off transcription of the *mPer* and *mCry* genes. As the levels of PER and CRY fall, CLOCK and BMAL1 will dimerize to restart *mPer* and *mCry* transcription restarting the 24-hour cycle.[5]

In addition to PER:CRY feedback inhibition, other processes contribute to the clock mechanisms. For example, PER2 (Figure 2) stimulates BMAL1 expression so that PER and BMAL1 expression are out of phase. Alteration in the phosphorylation status of PER proteins also influences PER stability and cellular localization. In Drosophila, the kinase double-time alters PER phosphorylation.[6] In mammals, casein kinase 1 epsilon[7] influences PER phosphorylation. Mutations in each of these kinases alter normal rhythmicity.

Evidence suggests that PER proteins also play a role in the photic regulation of clock phase. Following either photic or glutamatergic stimulation of the SCN, a cascade of calcium-mediated events is triggered, leading to activation of the transcriptional regulator CREB.[4] In turn, CREB binds to cAMP-response-element (CRE) sites within promoter regions to induce the expression of *mPer1* and *mPer2.* Alterations in PER protein expression then play a role in resetting clock phase.

Table 1
Homologous Genes in Drosophila and Mice that Play a Role in Circadian Clock Regulation

Drosophila	**Mouse**
period (per)*	mPeriod1 * mPeriod2 * mPeriod3 *
Timeless (tim)*	None
Time-out	mTimeless**
Cryptochromes (Cry)*	mCry1* mCry2*
clock*	mClock*
cycle*	mBmal1 (MOP3) mBmal2 (MOP9)
double-time*	casein kinases 1 epsilon (TAU)*

*mutation results in arrhythmic behavior
**mutation results in embryonic lethal

Adapted from Reppert and Weaver[1]

EXPRESSED RHYTHMICITY IN HUMANS AND OTHER MAMMALS

The rhythmic expression of intrinsic clock genes also drives the expression of clock-output genes, which communicate circadian phase to the rest of the organism.[4] This occurs as E-box elements, which are a binding site for PER, and which are present in promoter regions of other genes.[4]

Mutations in clock genes have been recognized in rodents with abnormal rhythmicity. Very recently, the first mutation of a human clock gene hPER2 has been discovered. This mutation results in the advanced-sleep phase syndrome that is characterized by very early morning awakening.[8,9] As other individuals with abnormal rhythmicity are identified, it is anticipated that additional clock gene mutations will be found.

Outputs of the circadian system have been widely characterized in human clinical studies. Notable examples include the sleep-wake cycle, daily rhythms in body temperature, and day-night rhythms in cortisol production. Day-night differences in gonadotropin, testosterone, growth hormone and thyrotropin secretion are also recognized.[10] Melatonin production by the pineal gland is also regulated by the SCN, with secretion occurring at night in proportion to the duration of darkness. In seasonal breeding species, changes in the duration of nocturnal melatonin production regulates the activity of the reproductive axis.[11] Melatonin does not appear to influence the human reproductive axis.[12] In humans, the duration of melatonin secretion is related to the length of days. The role of endogenous melatonin secretion in regulating SCN function is also unclear, as pinealectomized animals exhibit normal circadian rhythmicity and normal phase-shifting responses to light.[13]

Day-night differences are recognized for many homeostatic mechanisms such as body temperature, which has a nadir in the early morning hours. Cardiovascular function exhibits diurnal rhythmicity, as

does platelet function.[14] Rhythms in cognitive ability are recognized, and the productivity of shift workers and health care providers varies with the time of day.

There is also increasing recognition that the circadian cycle influences the pathogenesis of many illnesses. Myocardial infarctions and cerebrovascular events occur most commonly in the morning.[14] Croup and certain forms of asthma are associated with evening- hour exacerbations.[15] In some individuals, seizures are related to the time of day. Sudden infant death syndrome (SIDS) has a strong time related component, occurring most frequently in early morning hours.[16] However, we do not know if the circadian system plays a role in SIDS pathogenesis.

CIRCADIAN SYSTEM ABNORMALITIES

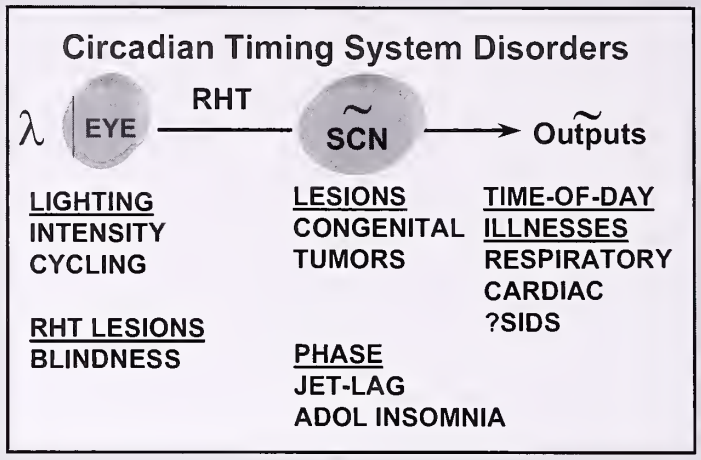
Since the circadian system exerts potent influences on human behavior and physiology, circadian system disorders will have overt clinical manifestations.[17] Circadian system disorders may be related to abnormal clock function or to abnormal entrainment of the clock (Figure 3).

When more than 90% of the SCN is damaged, arrhythmic behavior may result. Thus, congenital or acquired anterior hypothalamic lesions or tumors may result in the loss of expressed day-night rhythms on sleep-wake disorders.[18] Congenital central system abnormalities may also be associated with clock lesions, as we have discovered arrhythmic activity patterns in a child with septo-optic dysplasia.[19]

Clock disorders include abnormalities in circadian phase, which relate to the timing of expressed rhythmicity (e.g. the onset and offset of sleep-wake cycles) relative to the 24-hour day. Abnormalities of circadian phase occur when the "hands" of the endogenous clock are out of phase with the environmental light-dark cycle. One notable example of this phenomenon is jet lag, which occurs when circadian clock phase does not match that of light-dark cycle after changing time zones.

Another condition in which abnormal phase relationships occur is in delayed-sleep phase insomnia. In this condition that prominently affects adolescents, clock phase is delayed with resultant late sleep-onset and awakening times. Delayed-sleep phase insomnia should be considered when the individual does not fall asleep until after midnight and awakens late in the morning or in the afternoon. This condition becomes exaggerated when the effected individual is allowed to "sleep in" on weekends. Families with abnormally advanced circadian phase have also been described, some with hPER2 mutations, suggesting a strong genetic component for the setting of circadian phase.[8,9]

Figure 3



Schematic representation of the circadian timing system disorders (see text).

Entrainment disorders may result from inadequate retinal innervation of the SCN. In blind individuals without intact RHT function, the absence of photic information may result in impaired synchronization of endogenous and environmental phases. The circadian phase of such individuals will free-run, resulting in times when the individuals' sleep-wake cycles do not correspond with the light-dark cycle. Recent evidence shows that timed melatonin administration may help entrain the circadian phase of blind individuals who do not entrain to the 24-hour day. This helps synchronize sleep-wake cycles with the environmental light-dark cycle.[20] Surprisingly there are blind individuals who have intact retinal innervation of the SCN. In these individuals, environmental lighting will entrain the circadian clock so that endogenous rhythmicity is in phase with the light-dark cycle.[21] Unknown non-photic factors may also entrain circadian phase in blind individuals, as we have observed sleep-wake cycles in perfect synchrony with the light-dark cycle in individuals with anophthalmia.

Another cause of entrainment abnormalities is related to problems in environmental lighting conditions. If individuals are exposed to constant indoor lighting or darkness, or to low-intensity cycled lighting that is not potent enough to shift the clock (<200 lux), expressed rhythmicity will free-run. This situation can occur in constantly illuminated intensive care units where the patient's circadian phase will drift from that of care providers. This may result in perceptions of abnormal behavior. The interpretation of time-of-day dependent tests e.g., cortisol levels also will be inaccurate in this setting. Thus, to prevent free-running rhythms, cycled lighting of adequate intensity is needed.

DETECTING BIOLOGICAL CLOCK DISORDERS

A history of regular sleep and wake times in an individual is reassuring that the biological clock is functioning normally. The lack of regular sleep or awakening time may reflect abnormal clock function. Surprisingly, despite the socially disruptive effects of arrhythmic behavior, clock-related behavioral problems may not be brought to medical attention. Yet upon inquiry, families will give clear histories of abnormal activity patterns.

To assess clock function, diaries of sleep and waking times are useful. If the time the patient awakens and retires to sleep is consistent from day-to-day, this suggests normal clock function. However, if sleep patterns are irregular, or are out of synchrony with those of other family members, clock lesions may be present.

To provide objective assessments of behavior patterns, periods of rest and wakefulness can be assessed using monitors worn on the wrist that collect activity information for extended periods (actigraphy). Analysis of activity patterns collected over 2-3 week periods (actograms) can then be used to determine if there is normal rhythmicity or altered phase-relationships.

CHRONOTHERAPY

Over the past several years, considerable progress has been made in the treatment of biological rhythm disorders. Light has been recognized to regulate circadian rhythmicity in humans.[2] Exposure to bright light (10,000 lux) during the night is a strong stimulus that produces rapid shifts in circadian phase in humans.[2] Not surprisingly, light therapy is now being considered as a potential therapy for jet lag and other circadian phase disorders.

The concept that bright light resets the circadian clock is also important for night-shift workers. By providing an environment with bright light exposure during work at night and darkness during the daytime when the worker rests, it is possible to shift the endogenous circadian cycle to that of the work schedule.[22] Light therapy is also used in the treatment of certain forms of depression.[23]

Behavioral paradigms can be used to treat circadian-phase disorders. Delayed sleep-phase insomnia can be treated by progressively delaying sleep onset over several days until the patient's sleep-wake cycle is in phase with the desired time of day. Alternatively, imposing regular waking times each morning can help resynchronize circadian phase.

MELATONIN

Melatonin has received much attention as a "chronotherapeutic". Melatonin is an endogenous indolamine that is produced by the pineal gland at night in proportion to the duration of darkness.[24] In mammals, melatonin exerts its effects through specific high-affinity receptors that include Mel 1a (mel 1) and Mel 1b (mel 2) receptors.[25] These receptors consist of seven transmembrane spanning domains and couple with guanosine nucleotide binding proteins (G proteins).[25] In humans, the melatonin receptors have been identified in the SCN.[26] In non-human primates, melatonin receptors have been identified in the hippocampus, brainstem, thalamus and cerebral cortex.[27]

Melatonin has been touted as a therapy for a variety of conditions ranging from aging to cancer. Yet, as reviewed,[28] most of these claims have little credible scientific support. Melatonin, however, may have legitimate use in treating sleep disorders. Melatonin has well documented hypnotic properties, and is therefore effective in facilitating sleep onset.[29-31] The hypnotic effects of melatonin are most pronounced when melatonin is given in the evening.[32]

It has also been suggested that melatonin can acutely shift circadian phase and may have a role in treating clock disorders such as jet lag.[33] This issue remains controversial. Modest melatonin-induced phase shifts have been detected in some rodent species, but not in others.[34]

In humans, using the onset of melatonin secretion to mark circadian phase, it has been suggested that melatonin induces small shifts in circadian phase.[33,35] However, when primates are studied under rigorous conditions that are very difficult to achieve in humans, no phase shifting effects of melatonin are apparent.[32] These observations suggest that melatonin action in the treatment of jet lag[36,37] may be related to hypnotic effects, rather than phase-shifting properties.

Although melatonin may not acutely shift circadian phase,[32] melatonin administration at the same time each day may entrain free-running circadian phase. In blind individuals, nocturnal melatonin administration has been shown to entrain activity patterns to the 24-hour day.[20,37,38]

SUMMARY

Increasing evidence show that the circadian system exerts profound effect on human physiology. In parallel with increases in our understanding of the clinical importance of circadian biology, there has been an explosion in our understanding of the genetic

mechanisms that contribute to the workings of the circadian clock. Elucidation of abnormalities of the circadian system has also lead to the discovery of new clinical disorders that can now be identified and treated.

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Letter to the Editor

Ghrelin-induced obesity

The July issue of *Growth, Genetics & Hormones* (Vol. 17, p 34-35) contains a discussion of the ability of this 28 amino acid peptide to induce body fat accumulation in rodents.

But of great importance to students of human obesity is the observation that the lean weight of these obese animals was probably less, certainly not greater, than that of the controls. This finding puts such ghrelin-treated animals clearly at odds with the human state, for the latter usually have an increase in lean weight, most certainly not a decrement.[1] The only clearly documented exceptions to this rule are patients with the Prader-Willi syndrome[2,3] or Cushing's syndrome. With respect to body composition the human state differs from obesity induced by experimental hypothalamic lesions, from that of the "ob/ob" mouse, and the Zucker rat, all of which are characterized by a subnormal lean weight. Obviously, such animals, and those treated with ghrelin, cannot serve as models for human obesity.

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Gilbert B. Forbes, MD

Editor's Response: *Dr. Forbes in his talented analytical way has added significantly to the Abstract, Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism which dealt with studies in rats and not humans. With his astute commentary he reminds us that we should not necessarily project data obtained in rodents to humans. Neither of the Editors commenting on this article were so astute as to mention this most poignant point.*

Thanks very much, Dr. Forbes. The Editorial Board eagerly invites each reader to write and comment on pertinent points, ask questions or query us concerning what is published in Growth, Genetics & Hormones.

Robert M. Blizzard, MD
Editor-in-Chief

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Growth Hormone Treatment Enhances Bone Mineralisation in Children with Chronic Renal Failure (CRF)

Van Dyck et al report on bone mineralisation as determined by Dual Energy X-ray Absorptiometry (DEXA), of the whole body and lumbar spine prior, to and one-year after, the initiation of rhGH therapy in 10 pre-pubertal children with stable CRF. Inclusion criteria for the study included: (1) a height SDS of < -2 SD or a height velocity of < 25th percentile for age, (2) absence of growth hormone deficiency, (3) normal thyroid function, and (4) normal PTH levels. DEXA was used to measure total body mineral content (TBMC), lumbar spine bone mineral content (LBMC), total body mineral density (TBMD), and lumbar spine bone mineral density (LBMD), in patients and in a control group of 20 healthy children of similar age. DEXA was performed twice in the CRF patients and in the healthy controls. Body height was measured with a stadiometer and bone age was determined by TW2 method at the start and after one-year of treatment. Data were analyzed using Wilcox matched pairs.

Growth hormone treatment (1 unit or 0.3 mg/kg/week given in daily divided doses) was associated with an increase in median height velocity from 5.1cm/year (3.0-8.8 cm/year) to 10.6 cm/year (8.2-12.7 cm/year). Median creatinine clearance remained unchanged as did calcium, phosphorous, and intact PTH levels. There was, however, a marked change in serum alkaline phosphatase. This is a well-known phenomenon in different groups of patients treated with hGH and reflects osteoblastic activity. At the beginning of the study, the median bone age was delayed 1.9 years and increased 0.8 years over the duration of treatment. The patients' TBMC, TBMD, LBMC, and LBMD increased significantly after one-year of rhGH treatment ($p<0.05$ for each – see Table). When compared with height/age match controls, these values were not different at the start of treatment, nor at the end of treatment. Yet BMD, TBMD, and LBMD, significantly improved in patients over one year ($P <0.05$). When compared with age- matched controls, patients had lower TBMC and LBMC at the start of treatment and experienced a catch-up of LBMC to values similar to controls over the course of the year.

The authors note that there has been discrepancy in results from previous studies of various parameters of BMD in children with CRF treated with rhGH. They speculate that this might be explained by 2 factors - small sample size and selection bias. In the current study, findings demonstrate significantly improved BMD in children with CRF who are growth retarded. All subjects in the current study were on calcium supplements and their bone mineralisation was adequate for their height at baseline. The authors state that homogeneity of their results is most likely due to the homogeneity of the patients studied, that is pre-pubertal with severe renal disease from early years of life without signs of osteodystrophy. They conclude that rhGH treatment has a beneficial effect on BMC and BMD in pre-pubertal children with CRF. This was the finding of Lanes et al (*Horm Res* 1996;46:263-268).

Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

***Editor's Comment**: At first glance, the results of this short paper might not be appreciated as adding significantly to the information with regard to the effects of rhGH on children with renal disease. It is well known that BMC and BMD prior to puberty are important factors of similar measures in adults. Thus, any improvement which might be gained in the pre-pubertal years, could potentially be realized later in adult life. Indeed, the subjects in the Van Dyck study had indices of bone density comparable to those of height matched children at entry into the study and at the one-year follow up. What is significant is the increased BMC and BMD observed. These studies underline the importance of initiating rhGH therapy in children with CRF even when their absolute height deficiency is modest.*

William L. Clarke, MD

Table

Mineralisation parameter	Baseline	After 1 year rhGH	P
TMBC (g)	521 (144-944)	589 (225-1139)	< 0.01
TBMD (g/cm^2)	0.750 (0.672-0.888)	0.775 (0.681-0.995)	< 0.05
LBMC (g)	7.5 (3.8-15.7)	10.9 (5.9-18.0)	0.005
LBMD (g/cm^2)	0.475 (0.281-0.660)	0.525 (0.333-0.660)	< 0.01

Adapted from Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

Adipose Tissue is an Endocrine Gland Secreting Multiple Hormones

You Are What You Secrete is a summary and editorial by Saltiel in which he discusses two articles concerning adiponectin.[1] Saltiel emphasizes that our notion of the adipocyte as merely a cargo space for fat has undergone a dramatic change. We now know that adipose tissue is much more complex than previously thought, secreting proteins which include tumor necrosis factor (TNF)-α, leptin, adipsin, resistin and adiponectin known also as Acrp30 or adipoQ. These proteins perform diverse functions but share structural properties of cytokines, and are referred to collectively as "*adipokines*". Dynamic interactions occur between these proteins and dictate the extent to which insulin is sensed in its target tissues. In an article referred to by Saltiel, Berg et al[2] report that a single injection of adiponectin leads to a 2-3 fold elevation in its circulating levels, which precipitates a transient decrease in basal glucose levels. Similar treatment in ob/ob or streptozotocin - treated mice transiently abolishes hyperglycemia. This relative hypoglycemic effect is not associated with an increase in insulin levels. Moreover, in isolated hepatocytes adiponectin increases the ability of sub-physiological levels of insulin to suppress glucose production. Berg et al propose that adiponectin is a potent insulin enhancer linking adipose tissue and whole body glucose metabolism.

In the article by Yamauchi et al[3] the reversal by adiponectin of insulin resistance associated with both lipoatrophy and obesity is described. Yamauchi et al discuss the findings that recent genome-wide scans have mapped a susceptibility locus for type 2 diabetes to chromosome 3q27, where the gene encoding adiponectin is located. This group demonstrated decreased expression of adiponectin and its correlation with insulin resistance in mice models of altered insulin sensitivity. Adiponectin decreases insulin resistance in obese mice by decreasing triglyceride content in muscle and liver. Insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either given alone. Yamauchi et al concluded that decreased adiponectin production is implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy. Their data also indicate that administration of adiponectin might provide a novel treatment modality for insulin resistance in type 2 diabetes.

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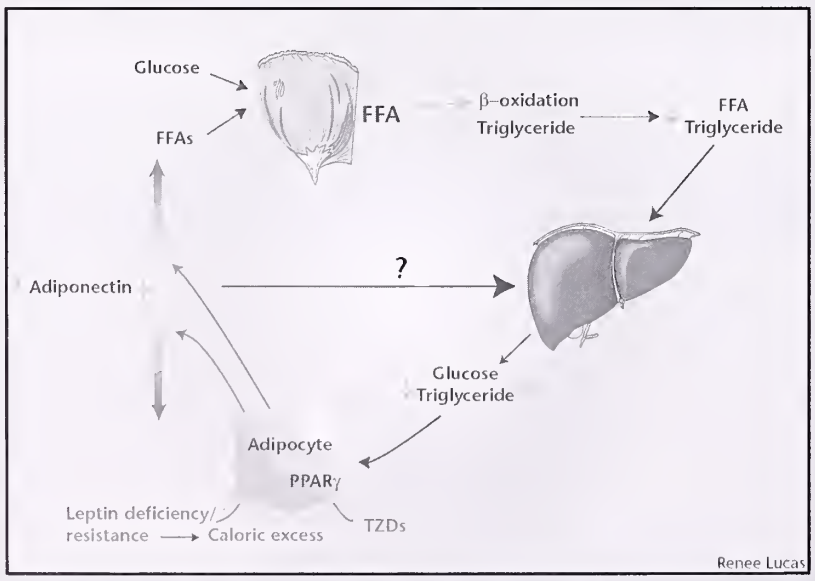
2. Berg AH, et al. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Med* 7:947-953,2001.
3. Yamauchi T, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Med* 7:941-946,2001.

Editorial Comment: *Adiponectin is a 247 amino acid protein whose expression in adipose tissue is depressed in obese animals. The plasma concentrations are low in these obese animals and also in obese humans, which is a pattern directly opposite to those of leptin, another adipocyte hormone. As discussed by Yamauchi et al, mice ingesting a high fat diet with increased fat accumulation had low tissue levels of adiponectin mRNA and low serum concentrations. Insulin resistance as reflected by hyperglycemia and hyperinsulinemia occurred.*

Figure



From Saltiel AR. You are what you secrete. *Nature Med* 7:887-888,2001.

A hypothetical model for the secretion and action of adiponectin. The synthesis and secretion of adiponectin is increased by activation of the nuclear receptor PPAR-γ, and reduced by caloric excess, presumably associated with leptin deficiency or resistance. Once released, adiponectin can directly increase fatty-acid transport, oxidation and dissipation in skeletal muscle, reducing the levels of intramyocellular lipids, thus improving insulin signaling. The protein can also increase the sensitivity of the hepatocyte to insulin, either through a direct action, or indirectly by lowering circulating lipids due to its action on muscle. Thus, administration of adiponectin can result in improved insulin sensitivity and glucose tolerance, and can correct hyperglycemia associated with obesity.

Administration of rosiglitazone, an inhibitor of peroxisome proliferator-activated receptor-γ which is an essential element for adipogenesis, increased adiponectin tissue mRNA values and also serum levels. Serum glucose was decreased as were serum levels of insulin.

In other mouse models of obesity (e.g. leptin receptor deficiency), administration of adiponectin lowered blood glucose and insulin values. In another mouse model, a lipodystrophic mouse without fat, serum concentrations of adiponectin were undetectable. Hyperglycemia and hyperinsulinemia were present. Administration of adiponectin lowered serum glucose and insulin levels. Both leptin and adiponectin were required in the lipoatrophic mice to restore serum glucose and insulin values to normal.

In the article by Berg et al, serum glucose concentrations were decreased with the administration of recombinant adiponectin to wild type, leptin deficient, and insulin deficient mice. Berg et al also demonstrated that adiponectin depressed hepatic glucose output in vitro which is thus the second physiological effect that might contribute to enhanced insulin sensitivity. In calorically restricted wild type mice, serum adiponectin concentrations were twice those of freely feeding animals suggesting that this adipokine may be important in prolonging the lives of such animals.

Thus, the data in these manuscripts indicate that adiponectin plays a key role in energy metabolism. It enhances insulin sensitivity by lowering serum and tissue triglyceride values, by uncoupling of oxidative phosphorylation in muscle, and by suppressing hepatic glucose output. In addition to the effects on energy metabolism, adiponectin depresses the inflammatory response that accompanies atherogenesis. Indeed, patients with coronary artery disease have lower plasma adiponectin concentrations than do controls. Adiponectin inhibits inflammation in part by suppressing proliferation of myelomonocytic progenitor cells by accelerating apoptosis. The potential utilization of adiponectin as a therapeutic agent for patients with obesity, diabetes mellitus types 1 and 2, hyperlipidemia, and/or atherogenic disorders is clearly enormous. A lead article regarding Adipose Tissue as an Endocrine Gland will appear soon in GGH.

Allen Root, MD

Genetic Basis of Stature – Genome-Wide Search for Genes that Influence Normal Adult Height

It is well known that short parents have short children and vice versa, and that variation in normal stature has a strong genetic component. However, despite many decades of interest in the genetics of stature, the relevant genes remain elusive. In fact, the genetics of most common traits and diseases in humans is not well understood. The principal explanation is that the geneticist's primary tool for mapping genes is of only limited power for finding genes that have modest effects, such as those that contribute to common diseases and variable traits such as stature. Recent advances in genomics, however, have made it feasible to apply genome-wide linkage analysis to such entities. Indeed, the group led by Eric Lander has used this approach to identify genetic linkage for adult height.[1]

In total, 2,327 individuals from 483 families were studied. Fifty-eight families resided in the Botnia region of Finland, 183 families were from other areas of Finland, 179 families were from southern Sweden and 63 families were from the Saguenay-Lac-St-Jean region of Quebec. They were originally ascertained to investigate other genetic traits. Males were older than 23.5 years and females older than 21.1 years to exclude individuals still growing. The original genotyping results that were based on average spacing of microsatellite markers from 6.5 cM to 12.5 cM depending on the study population, were reanalyzed using the variance-components method implemented in the GENE-HUNTER 2 protocol. The method uses nonparametric multipoint approaches to generate LOD scores for chromosomal locations that reflect the likelihood that genotype data being observed is due to linkage relative to the absence of linkage.

Evidence for linkage was detected in four instances. A LOD score of 3.85 was obtained for linkage at chromosome 6q24-25 in Botnia. A score of 3.40 was calculated for a marker located at 7q31.3-36 in Sweden. A LOD score of 3.35 was determined for markers at 12p11.2-q14 in Finland and a score of 3.56 was found in Finland for 13q32-33. The authors note that a companion study also detected linkage at chromosome 7 site.[2]

The authors are optimistic that they have identified chromosomal regions where genes that influence stature reside, especially on chromosome 7. However, they caution that definitive interpretation is difficult in the absence of confirmation of linkage in additional populations. They observe their results were inconsistent across the four study groups, but note that this is typical in linkage studies of common diseases. They discuss possible reasons for the inconsistency including variation in sampling, existence of genetic variation in different populations and statistical fluctuations and false positives due to unknown causes.

Editor's Comment: *An additional comment is pertinent to this topic. Many genes known to influence stature have been identified by searching for disease genes. Examples include genes that harbor mutations that cause chondrodysplasias and many other syndromes associated with short stature. They range from homeobox-containing genes such as SHOX to cartilage matrix protein genes, i.e., COL2A1 to transcription factor and receptor genes such as SOX9 and FGFR3, respectively. Similarly, mutations of Fibrillin 1 lead to tall stature in the Marfan syndrome. It seems likely that there are genes that influence stature that are not associated with disease. The approach used here should identify genes in both categories. It will be interesting to see what genes fall into the latter category.*

These papers are the first reported genome-wide studies of genetic linkage and stature. They probably represent the tip of the iceberg in terms of what will come as genetic markers become more dense, more populations are studied and analytical approaches become more sophisticated. As noted by Hirschhorn et al, identifying the genetic basis of variation in height raises important ethical issues as the potential for genetic engineering evolves. However, as they point out, a greater understanding of this subject could be beneficial in the contexts of establishing diagnoses and predicting adult stature of "short" children.

William Horton, MD

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2. Perola M et al. Quantitative-trait-locus analysis of body-mass index and of stature, by combined analysis of genome scans of five Finnish study groups. *Am J Hum Genet* 69:117-123, 2001.

Short Stature Homeobox-Containing Gene Deletion: Screening by Fluorescence in Situ Hybridisation in Patients with Short Stature

In an attempt to determine when to screen for SHOX gene deletion in subjects with short stature, Müsebeck and colleagues determined the frequency of SHOX deletions in 50 children with short stature. All children studied had a height < -2 SDS and 3 of the subjects also had the Madelung deformity (shortening and bowing of the radius with dorsal subluxation of the distal ulna and partial foreleg anomalies). Thirty-five of the 50 subjects had idiopathic short stature (ISS) accompanied by the absence of skeletal, endocrine, or organic symptoms and had no family history of short stature. Twelve subjects had upper limb abnormalities such as cubitus valgus. Two subjects had Léri-Weill dyschondrosteosis, and 3 had a congenital heart defect. Blood was analyzed by FISH process (Fluorescence In Situ Hybridization) for the SHOX deletion.

Microdeletions of the SHOX gene were not detected in any of the 35 patients with ISS. Of the 12 patients with additional upper limb abnormalities 5 (41.7%) displayed SHOX signals on only one sex chromosome. Of the 7 with short stature who displayed SHOX signals on 2 sex chromosomes, 3 had Madelung deformity and brachymetacarpia was present in the other 4. Point mutations of course are not picked up in the FISH technique. Molecular genetic methods will possibly detect point mutations in patients such as the 7 referred to above. Three patients with congenital heart defects did not carry SHOX deletions.

The authors state that their findings provide important guidelines for selecting patients for SHOX analysis. They state that children with ISS are unlikely to carry such a mutation of the SHOX gene. Indeed, other studies have shown the SHOX mutation in about 1% of all patients with ISS. The combination of short stature and skeletal abnormalities of the forearm, however, makes the SHOX mutation much more probable. The authors caution that a father carrying a SHOX mutation on the X chromosome could transmit these mutations to his son because of crossing over between the pseudoautosomal regions of the X and Y chromosomes during paternal meiosis.

Müsebeck J, et al. *Eur J Pediatr* 2001;160:561-565.

Editor's Comment: *SHOX gene deletion determinations have become increasingly popular in endocrine/genetic clinics evaluating children with short stature. Although, the number of subjects studied by Müsebeck et al is relatively small (n=50), their data are convincing. Apparently, SHOX gene determinations have little place in the evaluation of the child with ISS and should be reserved for those children who have deformities of the upper extremities even when those are very mild. Hopefully, data can be pooled in the future from numerous centers so that definitive guidelines for evaluation of SHOX gene determinations are more clearly defined.*

William L. Clarke, MD

Growth Hormone in Short Children: Beyond Medicine?

The increasing use of rhGH in short children with non-GH deficient (GHD) short stature, whether or not data support the efficacy of such treatment, may lead to its use being perceived as a cosmetic "enhancement". Drs. Bolt and Mul discuss the merits of the use of rhGH in such children and whether such treatment is "in the medical realm". Employing a disease-oriented model, rhGH would be administered only to patients with documented GHD or identified abnormal state (e.g., Turner syndrome) to restore health and normal functioning. The authors reject this approach because the differences between normal and abnormal growth and function are often indistinct. On the other hand, they also reject the "client approach" to prescribing of rhGH in which one would administer it "on demand" for any and all types of short stature including familial and idiopathic, because this approach might lead to "medicalization" of many perceived and apparent differences between individuals and make patients of otherwise healthy persons. Bolt and Mul believe the proper goal of medicine is to prevent or relieve suffering, both demonstrable and subjective, and advocate this approach to deciding when the administration of rhGH is or is not warranted. Suffering, while perhaps not always quantifiable, can be perceived by the family and physician. Thus, children with non-GHD short stature may be eligible for treatment with rhGH if s/he demonstrates present suffering or the potential for future suffering. They conclude that because the impact of short stature upon the functional status of normal adults is minor, treatment with rhGH "should take place in a research setting".

Bolt LLE and Mul D. *Acta Paediatr* 90:69-73,2001.

Editor's Comment: *The suffering individual is anguished, tortured, bitter and sad. However, it may not always be easy to identify the suffering short child. Firstly, the majority of short, otherwise normal children are brought to the office of the pediatric endocrinologist by their parents who are often more concerned about the height of their child than is the child himself. Thus, it is likely that it is the parent who is "suffering" rather than the child. Drs. Bolt and Mul do not address the issue of whether rhGH should be administered to a short child to alleviate parental suffering. Secondly, suffering related to short stature is seldom due exclusively to height, but reflects a constellation of behavioral, learning and social problems. As Macklin[1] points out in a companion commentary, the discomfort of the short-statured child may pale when compared to the physical suffering imposed by the numerous medical procedures that accompany treatment with, and the administration of rhGH. Although the "goal of medicine" involves all of the interrelated components delineated by the authors - disease-oriented, client-related, relief of suffering - this reviewer adheres to the precept that medicine is primarily a science and that medical decision making should be based upon valid scientific data. To date, there are limited and conflicting data relative to the growth promoting efficacy of rhGH therapy of the non-GHD short child and even fewer data concerning any psychosocial benefits of treatment.[2] Thus, I concur with the recommendation of Drs. Bolt and Mul that such treatment be undertaken in the context of a research environment.*

References

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Allen Root, MD

Extended Life-Span Conferred by Cotransporter Gene Mutations in ***Drosophila***

These investigators demonstrate that in the adult fruit fly, *Drosophila melanogaster*, heterozygous inactivating mutations in a newly identified gene *Indy* (for *I'm not dead yet* from the film "Monty Python and the Holy Grail") double the active, fertile, and fecund life span of this insect. *Indy* encodes a 572 amino acid sodium dicarboxylate cotransporter, a membrane protein that shepherds the uptake and re-uptake of di- and tricarboxylic acid intermediate metabolites (e.g., succinate, citrate) of the Krebs cycle across cell membranes of organs responsible for metabolism and storage of fat, glycogen, and protein (e.g., the liver in mammals). The investigators suggest that heterozygous loss-of-function mutations in *Indy* decrease the rate of absorption and utilization of metabolites, thus acting functionally to extend life span in a manner similar to that of partial caloric restriction.

Rogina B, et al. *Science* 290:2137-2140, 2000.

Editor's Comment: *Energy restriction has been demonstrated to extend life span in worms, mammals, and insects, but the mechanism(s) by which decreased calories does (do) so have not been identified. It may*

be that caloric restriction down regulates the expression of sodium dicarboxylate cotransporter(s) genes thus decreasing the rate of intracellular metabolism and consequently increasing cellular life. These observations suggest that perhaps some obese subjects possibly have gain-of-function mutations in one or another sodium dicarboxylate cotransporter that enhance intracellular intermediary metabolism leading to accumulation of fat, while other individuals (who can "eat a tone and never gain an ounce") may have a variant that impedes metabolism. The data also suggest that it may be possible to modify the activity of these cotransporter molecules chemically - opening a portal for treatment of a group of obese subjects.

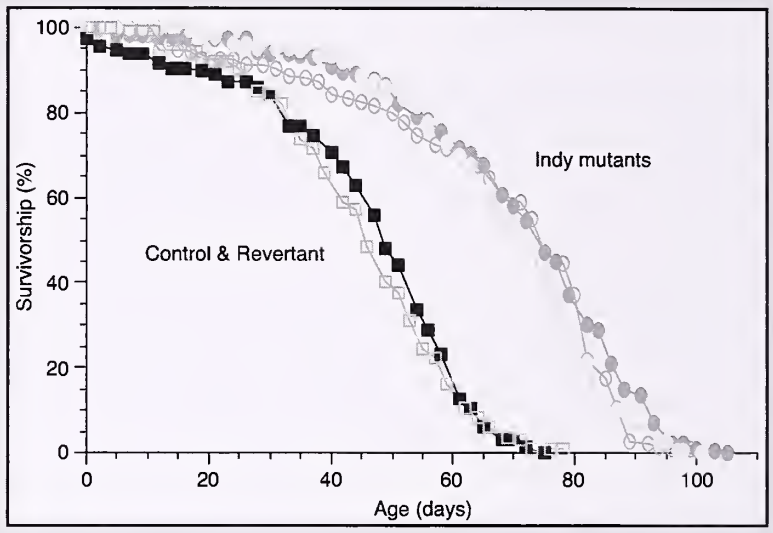
Pennisi E. Old files may hold secrets of aging. *Science* 290:2048, 2000.

Allen Root, MD

Figure

Life-span extension in *Indy* mutants. Survival curves of males heterozygous for three different *Indy* mutations, a precise excision of the P -element from Indy 302 (revertant), and an enhancer-trap control are shown. All flies were tested as heterozygotes over a wild-type Canton-S strain. The *Indy* mutants are *Indy302* (open white circles), *Indy206* (solid gray circles), and *Indy159* (strikethrough circles). The excision line (strikethrough squares) is one of four exact excisions (sequence confirmed) of the P element obtained by mobilizing the P element from either the *Indy302* or *Indy206* line, using delta 2-3 transposase. The control (solid black squares) is one of four other enhancertrap control lines from the same mutagenesis that generated *Indy302* and *Indy206*, tested as a heterozygote over Canton-S.

From Rogina B, et al. *Science* 290:2137-2140, 2000.



Insulin Resistance and Insulin-Like Growth Factors in Children with Intrauterine Growth Retardation

The authors recently proposed that when tissues in utero are chronically depleted of insulin and IGF1, but subsequently exposed after normalization of nutrient supply in postnatal life to increased levels, insulin resistance often develops. Carrying this thesis forward, they postulate that postnatal "catch-up" growth might, therefore, be associated with a higher risk of developing insulin resistance, especially when other risk factors such as genetic predisposition and/or obesity coexist.

To investigate this possibility, 49 children with IUGR (22 boys) with birth weight <10th percentile for gestational age were studied. Children with malformations and/or genetic disorders were excluded. Stature was corrected for mid-parental height. Children were divided into two groups according to their corrected height; specifically, those with corrected height z-score ≥0 and those <0. Insulin resistance was evaluated using OGTT, fasting glucose and insulin levels, and a G/I <6 to interpret insulin resistance. Thirty-nine percent (19/49) of the children with IUGR had a corrected stature >0 z-score and 61% had not reached their genetic height, as expressed as MPH z-score. Corrected stature at the age evaluated *correlated with* birth weight, whereas actual height was related to birth length, MPH and BMI. Twenty-two percent or 11 of 49 IUGR children had a G/I <6. The endocrine variables in children as divided on the basis of G/I <6 and >6 are provided in Table 1. All the parameters related to insulin resistance correlated with alanine aminotransferase (ALT) and gamma glutamyltransferase (γ-GT) levels. IGF system parameters were in the normal range and *correlated neither with* growth nor with insulin sensitivity.

The first aim of the study was to assess the prevalence of insulin resistance in children and adolescents with IUGR. The authors considered that insulin resistance was at a high prevalence since 22% of the children were so classified, and these data are consistent with previous studies reporting impairment in insulin sensitivity in children with IUGR. The second objective was to prove the *catch up growth hypothesis* that catch up growth induces insulin sensitivity. The data in this study suggest that catch up growth *is not* a risk factor. They further comment that the finding of high prevalence of insulin resistance *did not* show a significant influence over postnatal growth - is consistent with the intrauterine reprogramming previously postulated by the authors and is consistent with a genetic predispositioning determining both low birth weight and

insulin resistance. The authors also postulate that obesity may be an additional risk factor during childhood. One of the most important findings was the close relationship observed between insulin resistant parameters and liver function tests; this suggests that the liver might be a target organ of the reprogramming process. The authors did not find any indications that the IGF systems (IGF1, IGFBP-3, etc) are related to the insulin sensitivity status, at least during childhood. The latter data are in accord with those of at least two other authors.

Cianfarani S, et al. *Horm Res* 2001;55(suppl 1):7-10.

Editorial Comment: *The authors have provided excellent data on a large number of small for gestational age infants. I have not used the term intrauterine growth retarded children as in the title of the article, as I believe that term should be reserved for children who are <3rd percentile. I remain skeptical that one out of every 10 children is intrauterine growth retarded, which would be the case if one uses the 10th percentile as cutoff. The article as presented does not indicate to me what percentages of the children born <3rd percentile had insulin resistance. The authors and others are invited to comment to the Editor concerning which criteria are appropriate to use for determination of metabolic alterations in IUGR children, as much confusion now exists among data stated to be that of IUGR.*

Regardless of what I consider this limitation, the data are worthwhile and provide interpretations to postulated metabolic alterations in children who are small for gestational age.

Robert M. Blizzard, MD

Table

Anthropometric and endocrine variables in children with IUGR glucose/insulin (G/I) ratio <6 (n = 11) or >6 (n = 38)

	G/I <6(n = 11)	G/I >6(n = 38)	p
Age, years	10.3 ± 3.6	8.9 ± 3.3	n.s.
Birth weight, kg	2.16 ± 0.35	2.18 ± 0.38	n.s.
Birth length, cm	45.4 ± 2.8	45.8 ± 2.8	n.s.
Ponderal index, g/cm^3	0.002 ± 0.002	0.022 ± 0.004	n.s.
BMI, kg/m^2	18.5 ± 4.0	16.2 ± 3.9	n.s.
BMI, z-score	1.0 ± 2.6	-0.29 ± 1.8	n.s.
Height, z-score	-1.08 ± 1.29	-1.23 ± 1.3	n.s.
MPH, z-score	-1.4 ± 0.6	-0.8 ± 0.9	<0.05
Corrected stature, z-score	0.36 ± 1.1	-0.36 ± 1.3	n.s.
Fasting insulin, mU/l	12.4 ± 9.0	8.2 ± 3.3	n.s.
HOMA-IR	3.5 ± 1.0	1.5 ± 0.8	<0.0001
HOMA-β-CELL	180 ± 139	43 ± 90	<0.01
AUC_{ins}, mU/l	240 ± 113	164 ± 115	n.s.
Proinsulin, p*M*	9.6 ± 11.2	5.0 ± 4.4	n.s.
IGFBP-1, μg/l	83 ± 59	119 ± 50	<0.05
IGF-I, z-score	0.41 ± 2.8	0.47 ± 3.0	n.s.
IGF-II, z-score	0.56 ± 0.7	0.62 ± 0.9	n.s.
IGFBP-3, z-score	0.35 ± 0.7	0.23 ± 1.3	n.s.
IGF-I/IGFBP3 ratio	61.2 ± 23	63.5 ± 35	n.s.
AST, U/l	29.1 ± 8.0	27.4 ± 6.7	n.s.
ALT, U/l	27.4 ± 17.1	16.3 ± 7.2	n.s.
γ-GT, U/l	15.7 ± 6.6	11.7 ± 3.8	n.s.

ALT = alanine aminotransferase

AST = aspartate aminotransferase

AUC_{ins} = area under the curve of insulin during oral glucose tolerance test

BMI = body mass index

HOMA-β-cell = homeostasis model assessment β-cell function

HOMA-IR = HOMA for insulin resistance

IGF = insuline-like growth factor

IGFBP = IGF binding protein

IUGR = intrauterine growth retardation

MPH = midparental height

n.s. = not significant.

Adapted from Cianfarani S, et al. *Horm Res* 2001:55 (suppl 1):7-10.

The Molecular Basis of X-Linked Spondyloepiphyseal Dysplasia Tarda

The gene for X-linked form of spondyloepiphyseal dysplasia tarda has been identified as SEDT, a protein that apparently plays a role in endoplasmic reticulum-to-Golgi transport and involves subcellular localization of normal sedlin constructs. The protein is relatively small with 140 amino acids. It is located in the non-X-inactivated part of Xp22. This suggests that female carriers express sufficient normal gene to avoid the disease.

The present study looked at 36 unrelated cases and attempted to make phenotype/genotype correlations. Mutations could be found in 30 individuals. The 6 individuals in which mutations were not found either lacked a strong family history or convincing physical

features, and therefore, may represent other diseases. Twenty-one different gene mutations were observed among the 30 cases, and in those cases with several identical mutations, hypeotype analysis suggests that they arose separately and, therefore, do not represent a founder effect.

Intrafamilial variation was certainly observed; however, mutations occurring toward the five[1] end of the SEDL gene (mutations in Exons 3 and 4) resulted in kyphosis and scoliosis with severe pain early in life and with more debilitating types of complications. This was observed while mutations in Exons 5 and 6 resulted in milder clinical features.

Mutations were spread throughout the gene, including point mutations, splice alterations, insertions, deletions, and complex rearrangements. The most common type of mutation was a deletion. There was a 10 fold greater occurrence of deletions than would be expected. This may represent slippage during homologous recombination between the Y and X chromosome.

The SEDL phenotype may be explained by reduction in endochondral bone formation in the epiphysis, particularly in the vertebral bodies. A timely switch to up regulate the endogenous expression of a pseudo gene on chromosome 19 might provide gene therapy. The authors are undertaking a study of SEDL mutations in premature osteoarthritis.

Gedeon, AK, et al. *Am J Hum Genet.* 2001;68:1386-1397.

Editor's Comment: *When genes are identified for the chondrodysplasias, the possibility of making phenotype/genotype correlations and understanding the basic molecular biology are very enticing. This paper is a lovely demonstration of how a great deal can be learned in rare disorders by large international collaborations. This work hopefully will lead both to a better understanding of disease and to potential therapies.*

Judith G. Hall, OC, MD

Postnatal Malnutrition and Growth Retardation: An Inevitable Consequence of Current Recommendations in Preterm Infants?

Intake of adequate nutrients in preterm infants is difficult at best, and most often does not accomplish meeting the recommended dietary intakes (RDI). A nutrient deficit therefore accrues, leading to postnatal malnutrition and growth retardation. This study assesses the dietary intake in a prospective single observer design in 105 preterm infants with a body weight of < 1750 grams and a gestational age of < 34 weeks who were admitted to the Neonatal Intensive Care Unit over a 6 month period. Actual intake was subtracted from the recommended energy intake (120 kcal/kg/day) and protein (3 g/kg/day), and nutritional deficits were calculated. Infants were weighed on admission and throughout the hospital stay.

Nutrient intakes meeting current RDI's were rarely achieved during early life. By the end of the first week, cumulative energy and protein deficits were 406 +/- 92 and 335 +/- 86 kcal/kg and 14 +/- 3 and 12 +/- 4 g/kg in infants < 30 and those at > 31 weeks, respectively. By the end of the fifth week, cumulative energy and protein deficits were 813 +/- 542 and 382 +/- 263 kcal/kg and 23 +/- 12 and 13 +/- 15 g/kg. The z scores were –1.14 +/- .6 and -.82 +/- .5 for infants at < 30 and > 31 weeks. Stepwise regression analysis indicated that variation in dietary intake accounted for 45% of the variation in changes in z-score. The authors concluded that preterm infants inevitably accumulate a significant nutrient deficit in the first few weeks of life.

Editor's Comments: *This study clearly demonstrated that there is an accumulated nutrient deficit in preterm infants in an NICU setup. It also clearly suggests that the nutritional approach to the care of these infants needs to be re-thought, perhaps with a more aggressive approach, i.e. enteral or parenteral feedings. However, even early parenteral or enteral supplementation might be limited as these infants might not be able to tolerate it. A more aggressive enteral feeding is also hard to attain in the first few days of life, and it could lead to necrotizing enterocolitis or other adverse effects. The long-term consequences of this accumulated nutrient deficit may be important. It is generally assumed that poor growth in the preterm low birth weight infants primarily reflects inadequate nutrient intake, and in this study there was a 45% variation in growth related to such. Nonetheless, despite poor growth during the initial stages of life, most premature infants grow well thereafter and attain a normal height, unless there are other complications. Once the infant matures, the nutrient deficits are recouped and there is nutritional recovery with catch-up growth. However it should be kept in mind that nutrient deficits in early infancy might have other devastating consequences. The data from this study suggest that the clinician is in a quandary and that a more realistic picture regarding the quantity and quality of nutritional care in low birth weight infants needs to be re-thought.*

Embleton NE, et al. *Pediatrics* 107:270-272, 2001.

Fima Lifshitz, MD

The Land Between Mendelian and Multifactorial Inheritance

Burghes et al discuss the concept that genetic disorders can often be thought of as attributable to Mendelian and/or multifactorial triats. However, we now must consider other possibilities in classifying certain genetic syndromes. One such category has been classified as *triallelic inheritance*. The Bardet-Biedl syndrome, as published by Katsanis et al, is tagged as such. This article prompts a perspective commentary on genetics by Burghes et al.[1]

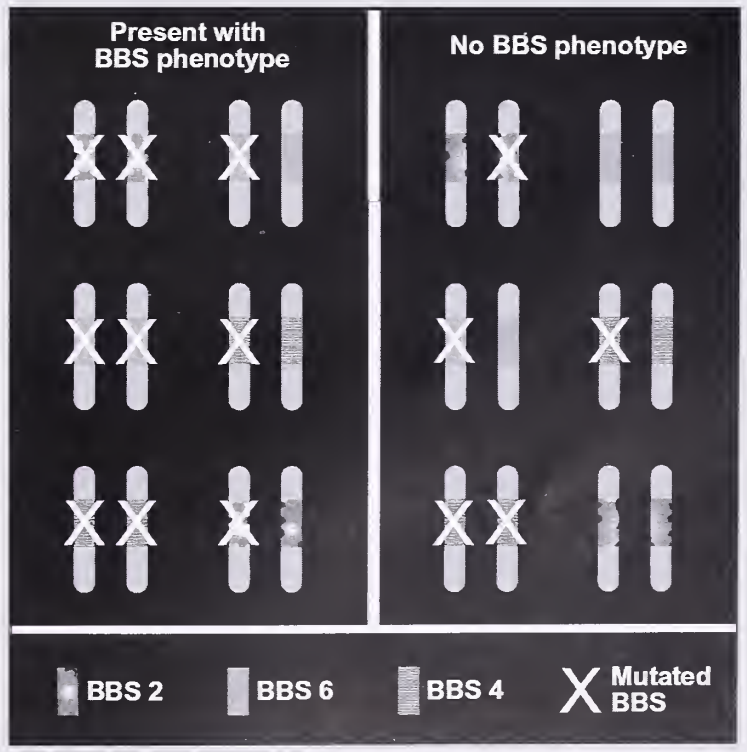
Although there has been spectacular success in identifying genes responsible for Mendelian inherited disorders, finding *susceptibility* genes involved in multifactorial diseases has been a struggle. How multiple genes interact to give the final phenotype of a multifactorial disease and what we might expect, remains an enigma. The land between Mendelian and multifactorial inheritance is inhabited by genes such as *modifier genes* and *redundant genes* that have many effects on the developing phenotype. Understanding the mode of action of these will help in determining how *susceptibility genes* may interact to give rise to a multifactorial phenomena.

Katsanis et al[2] report that mutations in two genes, rather than one, cause Bardet-Biedl syndrome (BBS). Katsanis points out that six BBS loci exist in humans. Three of these have been identified (BBS2, 4, and 6); the other three have not, as yet. Mutated genes have been identified in BBS2, BBS4, and BBS6 genes. Katsanis et al describe 11 subjects, out of a group of 163, who were genetically characterized with heterozygous or compound heterozygous mutations in BBS2, and three families with normal individuals who had the same two mutated BBS2 alleles. In three pedigrees the affected BBS patient had mutations of both BBS2 alleles and a mutation in one BBS6 allele. In one family the affected BBS patient had a mutation of one BBS2 allele and mutations in two BBS6 alleles. Thus, in four families mutations in three BBS alleles were demonstrated and apparently necessary for expression of the disease phenotype. Katsanis proposed that BBS may not be a single gene recessive disease, but a complex trait requiring three mutant alleles to manifest the phenotype. The phenotype of BBS includes pigmentary retinopathy, polydactyly, obesity, developmental delay, and renal defects. The figure illustrates the complex inheritance in Bardet-Biedl syndrome.

Editor's Comment: *The concept that mutations of genes on more than two alleles may be necessary for expression of a disorder is at odds with classical Mendelian transmission through dominant or recessive mechanisms, but is not incompatible with our understanding of diseases that appear to require multiple genetic and/or environmental factors for expression (e.g., diabetes mellitus, obesity, spinal muscular atrophy). Inasmuch as the majority of patients with BBS and mutations in BBS2 had normal BBS6, it is likely that these investigators will search for mutations in BBS4 (and BBS1 and 3 when they are identified) in this large group of BBS subjects. Since the phenotype of BBS is consistent despite the genotype, one suspects that the various BBS loci identified will be linked to one another in a metabolic process(es) that when interrupted leads to the disorder. Incidentally BBS6 is also mutated in patients with the McKusick-Kaufman syndrome of congenital heart disease, polydactyly, and transverse vaginal septum leading to hydrometrocolpos in females.*

Allen Root, MD

Figure



One mutation too many. Complex inheritance in Bardet-Biedl syndrome. Two allelic mutations in one BBS gene and a third mutation in another BBS gene are required for the disease phenotype to become manifest. The question is, what molecular interactions underlie the formation of the BBS phenotype? Three of the six BBS genes have been identified: BBS2 encodes a protein of unknown function, BBS6 is a putative chaperone, and BBS4 may be a glucosamine transferase. Three more BBS loci have been defined by linkage, although the genes have yet to be identified.

From Burghes AHM, et al. *Science* 293:2213-2214,2001.

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The Endocrine Function of Adipose Tissue

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INTRODUCTION

The traditional view of the adipocyte as a passive receptacle for storage and combustion of triacylglycerol is undergoing rapid change. It is now recognized that a variety of adipocyte and adipose stromal cell derived proteins act both locally and distally through autocrine/paracrine and endocrine effects to regulate fat cell differentiation, and sense and adjust systemic energy balance.[1] These adipokines are molecules that were previously identified to be derived from immune cells, while others, cytokines produced by adipocytes, were known to be involved in hemostasis, inflammatory response, vasoregulation, and steroid metabolism (Figure 1). Many of these proteins increase as fat mass accumulates and, thus contribute to the multiple morbidities of obesity. Increased activity of three of these, tumor necrosis factor, interleukin 6, and resistin, play a role in the development of the insulin resistance present in obesity. In contrast, other adipokines, like adiponectin and leptin, are insulin sparing through stimulatory effects on the beta oxidation of fatty acids in skeletal muscle.

The concept of "lipotoxicity" postulates that the accumulation of excess lipids in hepatocytes and skeletal muscle cells interferes with insulin signaling,[2] and the increased lipolytic activity of visceral fat contributes to this process by shunting fatty acids through the portal vein to the liver. Local overproduction of glucocorticoids in visceral fat ("Cushing's disease of the omentum") is also pathogenic. Increased activity of 11 hydroxysteroid dehydrogenase (11 HSD-1) raises adipose tissue cortisol levels, adversely partitioning fat into visceral sites and stimulating release of metabolically harmful adipokines.[2] Many of these adipokines also act centrally. Leptin, tumor necrosis factor (TNF) and interleukin (IL-6) enter the hypothalamus where they affect sympathetic tone, feeding behavior, thermogenesis, reproduction, and the activity of various hypothalamic-pituitary axes.Adipocyte

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Letter from the Editor:

The lead article in this issue covers a very current topic, one which pediatric endocrinologists may not be thoroughly familiar or are just beginning to incorporate into their sphere of interest (outline of article at *www.gghjournal.com*). However, it is a subject about which we all will be hearing a great deal more in the near future as pediatric endocrinologists become more involved in the care of obese patients. The epidemic of obesity is confronting our profession more than ever. Consequently, most readers of Growth Genetics and Hormones will benefit from having this article as a source for reference to broaden their knowledge about The Endocrine Function of Adipose Tissue. To serve this purpose the presentation of this article by necessity was very inclusive and written as an introduction to, and compilation about, the existence and known function of the many hormones outlined in the text. Dr. Diamond is to be commended for undertaking a difficult task and achieving the intended goal.

For the Editorial Board
Robert M. Blizzard, MD
Editor-in-Chief

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differentiation is controlled by the nuclear transcription factor, peroxisome proliferator activated receptor (PPAR)(Figure 2).[3] As energy surplus develops, adipocyte differentiation and lipid accumulation are inhibited through feedback loops of adipocyte-derived factors such as TNF, angiotensinogen (AGT), and resistin (for resistance to insulin). When energy deficit occurs, there is a decline in other adipocyte secreted proteins, such as adiponectin and leptin, and there is activation of trophic proteins such as acylation stimulating protein (ASP) and angiotensin II (AngII). These signal a drive to adipocyte formation and renewed triglyceride accumulation. Insulin is central to this process, promoting lipogenesis and energy storage. The development of insulin resistance which is concomitant with excessive accumulation of body fat may signify a physiologic counter regulation activated to maintain energy homeostasis of the adipocyte. As body fat accumulates beyond that needed for energy balance, and as adipose tissue is chronically exposed to excess dietary fatty acids and glucose, there are further maladaptive responses of adipokines, which result in insulin resistance, inflammation, hypertension, and endothelial disease.

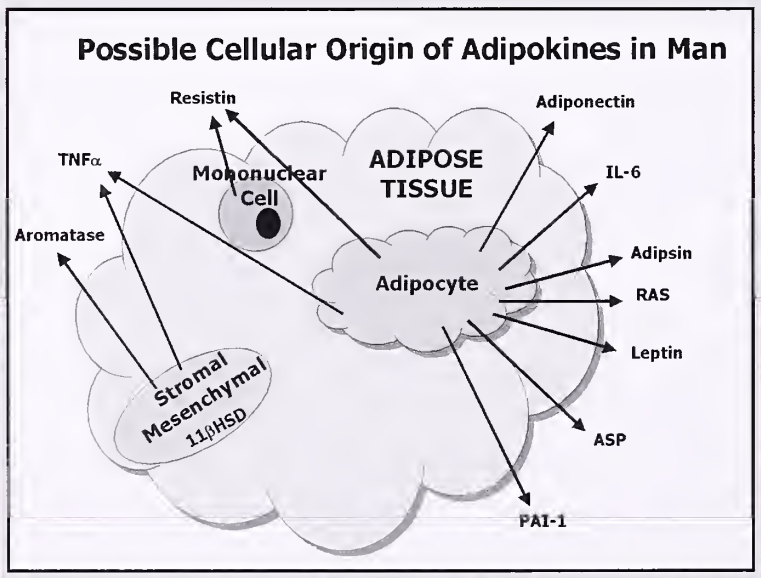
A review of the function and regulation of adipokines is made in this paper to facilitate the understanding by which obesity may contribute to the pathogenesis of the complications of this disease and of the alterations associated with this condition.

ADIPOKINES ASSOCIATED WITH INSULIN SENSITIVITY

Adiponectin

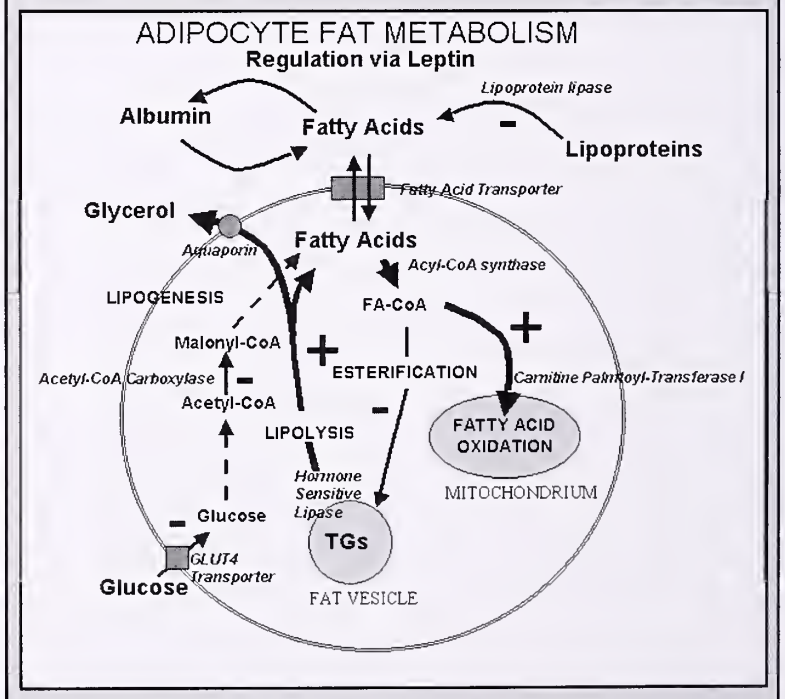
Adiponectin [Adipocyte complement-related protein (ACRP)], a soluble defense collagen, which is a circulating matrix-like protein, is expressed abundantly and exclusively in white adipose tissue.[4] Adiponectin appears to be an endogenous anti-inflammatory and anti-atherogenic factor that is protective against insulin resistance and macroangiopathy.[5] Its serum concentrations are reduced in obese mice and humans and rise following weight loss. This suggests that adiponectin plays a negative feedback role in fat storage.[6] Levels are lower in men compared to women and in individuals with obesity, type II diabetes, and coronary artery disease as compared to healthy subjects.[7] Its concentrations correlate with the insulin sensitivity state and with steady state plasma glucose, and rise in response to insulin. The protein is not an insulin sensitizer, however, but protects insulin action by accelerating beta oxidation of free fatty acids in skeletal muscle.[8] Intravenous administration of the "fat burning" c-terminal globular region of AdipoQ, the mouse homologue of adiponectin, reduces circulating free fatty acids and diet induced weight gain and corrects both hyperglycemia and hyperinsulinemia in genetically obese animals.[9] Hypoadiponectinemia may also

Figure 1



Adipose tissue signaling proteins may be produced in fat by adipocytes, mesenchymal/stromal cells, and mononuclear cells. They act through paracrine/autocrine and endocrine effects and several cross the blood brain barrier into the hypothalamus. The concentrations of these adipokines rise with increasing fat mass with the exception of adiponectin, which declines. IL-6 (interleukin-6), RAS (renin angiotensin system), ASP (acylation stimulating protein), PAI-I (plasminogen activator inhibitor-1), TNF (tumor necrosis factor), 11 HSD (11-hydroxysteroid dehydrogenase).

Figure 2



In the adipocyte, leptin inhibits lipogenesis by negative regulation of acetyl-CoA carboxylase, the rate limiting step in fatty acid synthesis. Leptin stimulates hormone sensitive lipase, which hydrolyzes triglycerides, diacylglycerols and cholesterol esters and downregulates lipoprotein lipase, reducing fatty acids available for lipogenesis. Glucose uptake is inhibited by leptin, reducing the availability of glycerol-3-phosphate required for triglyceride synthesis.

contribute to the insulin resistance of lipoatrophic animals, explaining the apparent paradox of glucose intolerance in both obese and fat depleted models. Adiponectin is highly regulated during adipocyte differentiation and may mediate some of the insulin-sensitizing effects of thiazolidinedione (TZD) binding to PPAR. Clinically, treatment of insulin resistant human subjects with TZDs significantly increases plasma adiponectin concentrations without affecting body weight. Additionally, adiponectin suppresses phagocytic activity, macrophage release of TNFα, and transformation of macrophages to foam cells in vitro. It also is deposited in vascular smooth muscle to protect vessel walls and thereby modulates the disease risks of coronary artery disease.[10]

Leptin

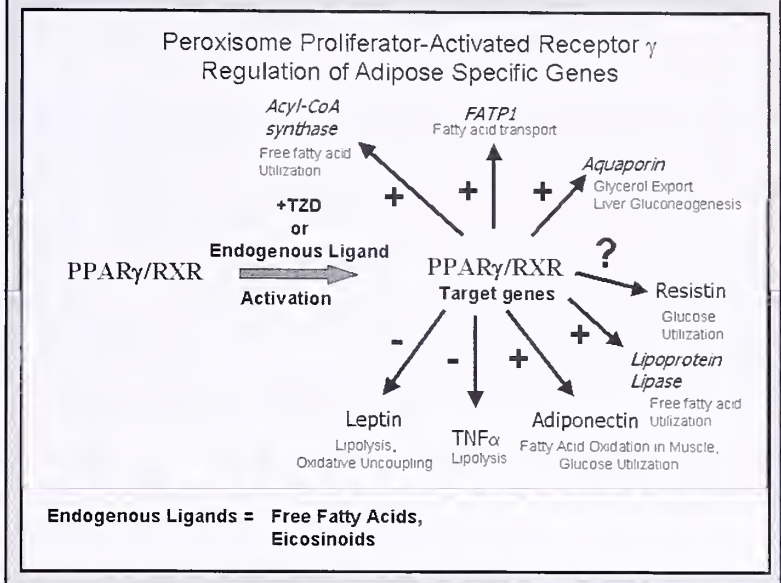
Leptin is a 16 kDa adipocyte-derived cytokine synthesized and released from fat cells in response to changes in energy stores and in systemic energy balance. Leptin's primary physiologic function is the defense of body fat. Declining levels in adipose tissue and serum signal the presence of energy deficit to the brain. Leptin circulates partially bound to plasma proteins and enters the CNS by diffusion through capillary junctures in the median eminence and by saturable receptor transport in the choroid plexus. In the hypothalamus leptin binds to long receptor isoforms which stimulate anorexigenic and inhibit orexigenic peptides.[11,12] Leptin also increases sympathetic nervous system activity and energy expenditure.[13] Adipocyte levels of leptin mRNA and protein correlate closely with both circulating leptin values and total body fat.

Leptin's lipolytic role in adipocyte metabolism is shown in Figure 3. Leptin reduces the levels of intracellular lipid in skeletal muscle, liver and pancreatic beta cells, thereby improving insulin sensitivity. In muscle this insulin sensitizing effect is achieved through inhibition of malonyl CoA, permitting increased transport of fatty acids into mitochondria for beta oxidation. These changes are partially mediated by central sympathetic activation of adrenergic receptors.[2]

Leptin synthesis is both constitutive and hormonally controlled. It is influenced by the state of energy reserve, and it is modulated by the sympathetic nervous system through an inhibitory feedback loop. Both adipocyte size and location dictate leptin production, although the mechanism(s) of these paracrine/autocrine modulated effects remain largely undefined. Larger fat cells contain more leptin than smaller ones and subcutaneous fat releases more leptin than visceral fat.[14,15] Several experimental findings suggest that glucose is an important regulator of adipocyte leptin release.[16] In cultured rat adipocytes, glucose inhibitors block leptin synthesis. In man, glucose infusion attenuates the rapid fasting decline of leptin. The hexosamine biosynthetic pathway into which 2-3% of cellular glucose uptake enters may mediate this link. Exposure of isolated subcutaneous adipocytes to UDP-N-acetylglucosamine (an end product of hexosamine biosynthesis) increases leptin release. Its inhibition reduces glucose-stimulated leptin release and ob gene expression. UDP-N-acetylglucosamine levels in human subcutaneous adipose tissue correlate significantly with both body mass index (BMI) and serum leptin levels.[17]

Insulin stimulates the secretion of leptin when administered to human subjects for several days. In adipocytes from rat white adipose tissue, leptin is present in the endoplasmic reticulum in the absence of insulin, whereas it localizes into the plasma membrane following insulin treatment.[18] Glucocorticoids, whose effects may be primarily permissive, induce leptin synthesis in vitro and in vivo, with greater responsiveness in obese as compared to lean individuals.[19,20] Females produce more leptin than males when matched for age, weight and body fat. This is probably related to gender differences in fat depots and to the leptin-suppressive effects of testosterone. At birth, the leptin concentrations in umbilical cord blood from girls are double those present in boys.[21] Pulsatile

Figure 3



Peroxisome proliferator-activated receptor (PPAR), a member of the nuclear hormone receptor superfamily, is a ligand-activated transcription factor. PPAR is central to adipocyte function, promoting differentiation of preadipocytes to mature fat cells. PPAR knockout (-/-) mice have no detectable adipose tissue. PPAR is endogenously regulated by heat shock proteins, fatty acids and prostaglandin J derivatives. Its DNA binding requires heterodimerization with 9 cis-retinoic acid receptor (RXR) followed by interaction with peroxisome proliferator response elements (PPRE) on adipocyte target genes. The insulin sensitizing thiazolidinedione (TZD) drugs are PPAR ligands whose effects on insulin signaling are mediated in part through PPAR stimulation or inhibition of intracellular adipokines.

leptin secretion correlates with female sex hormones. However, there are conflicting data regarding the influence of ovarian sex steroids on leptin release.[22,23] Other controlling factors are listed in the addendum.[24-26]

The prevailing evidence of the physiologic role of leptin suggests that it is an anti-obesity hormone, but this concept must be reconciled with the inability of high endrogenous leptin levels to prevent most obesity. It appears that in the majority of cases there may be leptin resistance mediated by inhibition of leptin signaling, thereby altering the dominant role of this hormone as a signal to switch between fed and fasted states.

ADIPOKINES ASSOCIATED WITH INSULIN RESISTANCE

Resistin

Resistin is a 12.5 kDa cysteine-rich adipocyte secreted protein which was identified during the screening for genes induced during adipocyte differentiation. This adipokine is down regulated by TZDs. It also is known as Fizz3 (for found in inflammatory zones). Worthy to note is that resistin is one of a family of similar molecules present in fat. Resistin administered to wild type animals induces insulin resistance, but in the obese-insulin resistant mouse it restores normal insulin sensitivity.[27] In morbidly obese humans, resistin mRNA from adipose tissue samples is increased as compared to that in lean controls.[28] However, a number of clinical and experimental observations suggest that resistin may not be the long sought major link between human obesity and insulin resistance.[29]

Tumor Necrosing Factor

TNFα is a multi-potential cytokine with diverse immunologic functions. Initially it was described as a cause of tumor necrosis in septic animals and was associated with cachexia-inducing states, such as cancer and infection.[30] In obese humans TNFα and its receptors (TNFR1 and TNFR2) are synthesized and secreted in increased amounts by adipocytes and stromovascular cells. Their autocrine effects contribute to the insulin resistance of obesity and diabetes;[31] TNFα inhibits insulin action by down regulating GLUT4 mRNA in fat and muscle. It also reduces insulin receptor autophosphorylation and phosphorylation by decreasing insulin receptor substrate-I. Circulating free fatty acids (FFA) increase from the lipolytic effects of TNFRI.[32] TNFα induces lipolysis which is blocked by PPAR ligands in insulin resistant animals.[33] In man, TNFα concentrations decline with weight loss and treatment with TZDs. The administration of TNFα causes hyperinsulinemia without hypoglycemia.[34]

TNFα also has important effects on the hypothalamus.In rats, intravenous or intracerebroventricular injection of TNFα stimulates ACTH secretion through eicosandoid cyclooxygenase mediated release of CRH and inhibits secretion of TSH.[35] Thus, TNF appears to have a net effect in prevention of obesity through the inhibition of lipogenesis and increased lipolysis with facilitation of adipocyte death via apoptosis.

Interleukin-6

In man, ~30% of circulating IL-6 originates from adipose tissue.[36] Concentrations are higher in visceral fat as compared to subcutaneous fat. They increase with obesity and are stimulated by TNF and IL-1.[37] Elevated levels are associated with increased risk of coronary artery disease, athero-sclerosis, and unstable angina.[38] Acting on the liver, IL-6 is a primary stimulant of acute phase reactants, such as C-reactive protein, fibrinogen and haptoglobin, thus contributing to a hypercoagulable state. Importantly, IL-6 also promotes the release of endothelial adhesion molecules[39] and adversely affects insulin sensitivity by inhibiting GLUT-4, hepatic glycogenesis, and lipoprotein lipase. The resultant lipolysis increases non-esterified free fatty acids (NEFA) which impedes nitric oxide mediated endothelial vasodilation.[40]

IL-6 receptors are present in the hypothalamus where IL-6 stimulates thermogenesis and satiety by increasing prostaglandin synthesis and release of corticotrophin releasing hormone (CRH).[41] It remains to be determined whether IL-6 is a link between obesity and thromboembolic complications.

ADIPOCYTE PROTEINS AND LIPID METABOLISM

Adipsin

Adipsin (ADIPocyte-trypSIN) is a 24-kDa adipocyte secreted protease with close homology to human complement D. This protease is required for the synthesis of acylation stimulating protein (ASP) (vida infra), which is described below and which is an important mediator of lipogenesis. Although adipsin concentrations are reduced in rodent models of obesity, paradoxically they are increased in humans with excess adiposity;[42] for example in obese Pima Indians serum adipsin levels are 45% higher than in non-obese Pimas or other controls. In subjects with anorexia nervosa the adipsin levels are low and rise during refeeding. Insulin stimulated adipsin release is mediated by ADP-ribosylation factor 6 (ARF6) which acts on endocytotic and recycling pathways in the adipocyte; therefore being an important protein in fat metabolism.[43] Adrenalectomy of ob/ob mice raises circulating adipsin levels; and corticosterone replacement reverses these changes. Adipsin secretion also is stimulated in animals by sympathomimetic agents, but not by cold stress.[44]

Acylation Stimulating Protein (ASP)

ASP is a 76-amino acid protein that stimulates fatty acid uptake and esterification into triglycerides. Retinoic acid (transported as retinyl ester by transthyretin and chylomicrons) stimulates the C3 gene leading to increased postprandial production of ASP.[45] Up to a quarter of patients with coronary artery disease have elevated concentrations of ASP. Hyperapobetaliproteinemia, a familial dyslipidemia characterized by increased hepatic release of LDL and VLDL, may result from impaired adipose tissue actions of ASP.[46] In the ASP-knockout mouse, postprandial triglyceride clearance is delayed and weight gain decreased. Like insulin and additive to it, ASP promotes movement of glucose transporter vesicles in cell membranes in adipose tissue and muscle by activation of the diacylglycerol/protein kinase C pathway.[47] This provides glucose substrate for glycerol-3-phosphate synthesis of fatty acids and triglycerides. Thus a deficit of ASP results in increased post prandial fatty acids and decreased weight gain and triglyceride synthesis.

Aquaporin Adipose (AQPap)

AQPap is an adipose specific glycerol channel gene abundantly and exclusively expressed in white adipose tissue. AQPap regulates glucose homeostasis by controlling the flux of glycerol into hepatic gluconeogenesis. In wild-type mice, AQPap expression increases during fasting, and declines with refeeding. This takes place through insulin action at the AQPap promoter's negative insulin response element (IRE).[48] AQPap is increased in adipose tissue from TZD treated mice and reduced in PPAR +/- heterozygous knock-out rodents.

ADIPOKINES & HEMOSTASIS

Plasminogen Activator Inhibitor-1 (PAI-1)

PAI-I, which is synthesized in the liver and in adipose tissue regulates thrombus formation by inhibiting the activity of tissue-type plasminogen activator, an anti-clotting factor. PAI-I concentrations in serum increase in proportion to visceral adiposity and are entrained by adipocyte size and lipid content.[49] Omental tissue explants secrete significantly more PAI-I than subcutaneous tissue from the same subject.[50] Increased PAI-I levels are found in patients with coronary artery disease and following myocardial infarction, while levels decline with caloric restriction, exercise, weight loss, and treatment with metformin.[51]

THE ADIPOCYTE RENIN-ANGIOTENSIN SYSTEM (RAS)

A renin-angiotensin system (RAS) located in the intra adipose tissue regulates fat cell mass and energy stores through paracrine/autocrine effects on adipocyte differentiation and lipid storage. Angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE), angiotensin II (AngII) and its receptors (ATI, AT2), and the non-renin-angiotensin enzymes chymase, cathepsins D and G, and tonin, are all expressed by adipose tissue.[52] Plasma AGT, renin activity and ACE correlate positively with body mass index while adipose tissue AGT expression correlates significantly with waist-to-hip ratio in man.[53] Adipose tissue AngII controls terminal differentiation of preadipocytes to adipocytes through the action of prostacyclin (PGI_2) and regulates adipose tissue blood supply. Adipose tissue AGT also influences adipocyte vascular resistance, but negatively regulates fat mass by decreasing lipogenesis. Ang II and AGT receptors are found in higher concentrations in visceral fast as compared to subcutaneous adipose tissue in both lean and obese individuals.[54] Glucocorticoids in the presence of insulin, and beta-adrenergic stimulation, and nutritional changes modulate adipoctye AGT gene expression.[55] In man, the role of the adipocyte RAS in the relationship between obesity and hypertension remains to be further defined.[56]

ADIPOSE AROMATASE AND INTRAADIPOSE GLUCOCORTICOIDS

Aromatase

Sex steroids are not synthesized de novo in fat, but are formed by the action of stromal enzymes on adrenally derived precursors. In human adipose tissue aromatase activity is principally expressed in mesenchymal cells of undifferentiated preadipocyte phenotype.[57] P450arom, a heme protein product of the CYP I9 gene, converts androstenedione to estrone. Estrogen production in fat rises as body weight increases and as subjects age.[58] Importantly, adipose tissue-derived estrogens partition fat to subcutaneous and breast tissues, while androgens promote central or visceral fat accumulation.[59] Aromatase activity varies significantly by region, with greater expression in adipose tissue from buttocks and thighs compared to that from abdomen and breasts.[60] In vitro, aromatase expression is stimulated by glucocorticoids in the presence of serum, and by class I cytokines. TNF increases aromatase expression in adipose stromal cells exposed to dexamethasone; leptin has little effect.[61] In the aromatse deficient ArKO mouse which lacks a functional Cyp 19 gene, there is a progressive accumulation of intra-abdominal fat and reduced lean body mass.[62]

11- HYDROXYSTEROID DEHYDROGENASE

11-hydroxysteroid dehydrogenase (11 HSD-1), which regenerates metabolically active cortisol from cortisone in man and corticosterone from II dehydrocorticosterone in mice, is increased in adipose tissue from obese

subjects. Adipose tissue corticosterone was overproduced by 30% in a transgenic (Tg) mouse that modestly over expresses 11 HSD in all its adipose tissues. The Tg male animals disproportionately accumulated visceral fat in adipocytes which were three times the size of those of control animals. The mice became hyperphagic, hyperglycemic, and hyperinsulinemic, had reduced levels of adiponectin and uncoupling protein-I, and had increased concentrations of leptin, TNF, angiotensinogen, lipoprotein lipase, and portal free fatty acids. This clinical and biochemical pattern mimics the human "metabolic syndrome".[63] In humans thiazolidinediones significantly reduce 11 HSD-I mRNA in vitro and in vivo, and preferentially reduce visceral fat.[64]

OTHER ADIPOCYTE PROTEINS

Metallothionein is an adipocyte secreted low molecular weight metal binding and stress response protein which may function to protect fatty acids from oxidative damage.[65] The metallothionein genes (MT-I, MT-2) are expressed in adipocytes early in their differentiation process. In vitro, MT-I transcription is stimulated by dexamethasone, forskolin and bromo-cAMP, and to lesser extent by insulin and leptin. Fasting-induced adipose factor (FIAF), a circulating fibrinogen-angiopoietin-related protein, is an adipocyte derived protein which increases during caloric deprivation and interacts with PPAR.[66] Lipoprotein lipase, cholesteryl ester transferase, apolipoprotein E, and retinol binding protein are other adipocyte proteins important for lipid metabolism which are under study.

CONCLUSION

The mechanisms by which obesity contributes to insulin resistance, hypertension, and endothelial disease are among the most important scientific questions facing medical investigators today. Research into the function and regulation of adipocyte signaling proteins, adipocyte differentiation, and the control of fat partitioning will likely result in further insight into these mechanisms and the discovery of targeted therapies for treatment of obesity and obesity related diseases.

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Addendum (re Leptin)

Many regulatory sites for leptin are found within the ob gene promoter, including cyclic AMP and glucocorticoid response elements, as well as loci for CCATT/enhancer and SP-I binding.[24,25] Thiazolidenediones reduce leptin mRNA in adipocyte 3T3-LI cells through negative PPAR effect at the leptin promoter.[26] Peripheral leptin administration activates suppression of cytokine signaling-3 (SOCS-3) which is co-expressed in hypothalamic nuclei with long-form leptin receptors. Increased SOCS-3 expression in vitro has been shown to blunt leptin receptor signal transduction by inhibiting JAK activity. SH2-containing phosphatase 2 (SHP-2) also blocks STAT-3 mediated leptin transcription. Moreover leptin is negatively regulated by the sympathetic nervous system via beta-2 and beta-3 catecholaminergic input at the adipocyte. The increased sympathetic enervation in visceral fat may thus partly explain its reduced leptin content compared to subcutaneous fat tissue. Infusion of isoprenaline or epinephrine in man acutely suppresses leptin release, as does cold exposure. Growth hormone, thyroid hormone, and melatonin have also been shown to decrease leptin secretion.

Abstracts from the Literature

Celiac Disease in Children with Autoimmune Thyroid Disease

This study was designed to test for the presence of celiac disease among children with autoimmune thyroid disease (ATD). Ninety patients (78 females) ages 1.8 to 17.3 years with ATD were studied; 20 of them had Graves' disease, and 16 had other associated conditions i.e. alopecia (4), vitiligo (2), juvenile rheumatoid arthritis (2), autoimmune hepatitis (2), Down's syndrome (1) and other miscellaneous autoimmune alterations (5). Screening for IgA antiendomysium antibodies (EMA) and HLA typing for Class I and II DQA1 and DQA2 heterodimers were done. There were 7 patients with positive EMA; an intestinal biopsy in these patients revealed intestinal villi alterations, with partial or total atrophy, crypt hyperplasia and intraepithelial lymphocytes. Clinically, one of the celiac disease patients had iron deficiency, one had diarrhea, and one had short stature, while the others were asymptomatic. A significant positive correlation was present for celiac-susceptible heterodimers in the patients with celiac disease. The authors concluded that screening for celiac disease should be done on all patients with ATD.

Larizza D, et al. *J Pediatr* 2001;139:738-740.

Editor's Comments: *This report is one more in the recent literature documenting the presence of celiac disease among patients with endocrinopathies. The prevalence of celiac disease in patients with ATD was 7.7% which is higher than that observed in other studies of adults with ATD, and of course much higher than the 1% reported in normal populations.[1-3] In Vol 17 No 2 of Growth Genetics & Hormones, I abstracted and commented upon the article describing the presence of celiac disease in 4.6% of children with type I diabetes.[4] Celiac disease was a significant factor in the development of hypoglycemia complicating the course of the diabetic illness. The presence of celiac disease in the patients in this study, as well as those in other reports, was without clinical evidence of malabsorption and the patients were largely asymptomatic. Nonetheless, it has been suggested that the presence of unidentified celiac disease could play a role in the development of autoimmune disorders, and the prompt diagnosis and treatment of this disease could prevent the onset of other alterations.[5] The availability of an accurate, sensitive and specific test (IgA antiendomysium antibodies) to screen for celiac disease should not be overlooked by Pediatric Endocrinologists who in my opinion should test all patients with autoimmune endocrine disorders regularly for antibodies reflecting the presence of celiac disease.*

Fima Lifshitz, MD

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The Role of Fetal-Maternal Microchimerism in Autoimmune Disease

Over the last 4 or 5 years, more and more diseases are described in which fetal cells are found at the site of autoimmune maternal disease and more recently maternal cells are being found at the site of newborn destructive ("graft-versus-host") diseases. Many diseases including systemic sclerosis and fetal dermatomyostis have now been attributed to fetal-maternal microchimerism. The report by Klinschar et al adds to the evidence that Hashimoto's thyroiditis includes fetal microchimerism in the fetal thyroid gland. These authors took thyroid gland specimens, extracted DNA, and then used Y probes to look for evidence of male cells in the maternal thyroids. They specifically used thyroid glands from women who had male children, and found evidence of male microchimerism in half the specimens. Among the controls (nodular goiter), only 1/25 specimens had evidence of Y chromosome microchimerism.

The importance of this observation is related to the question of whether the fetal cells can be a cause of autoimmune diseases since there is an excess of thyroid autoimmune disorders in females. The molecular techniques presently used look for Y DNA probes in females and female cells in males. The new molecular techniques allow this sort of recognition. It seems likely that all of us carry some maternal stem cells and that women who have been pregnant carry fetal cells, which can respond to damage and stress. What is not clear is whether the fetal cells are the cause of auto immunity or simply represent a stem cell response to injury.

Klintschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.

Editor's Comment: *It will be important to look at multiple tissues for fetal cells. It appears that pregnancies which have been complicated are more likely to have fetal cells in circulation. Thus, pre-eclampsia and anueploidy are known to have increased trafficking between mother and fetus. In addition, loss of co-twins can predispose to microchimerism. Keep your eyes open for more work in this area since it is highly likely that additional papers will try to discriminate the source of the cells, and determine the time at which they would have migrated to specific tissues.*

Judith G. Hall, OC, MD

Table

Number of children, sons, and daughters in Hashimoto patients with and without detectable microchimerism

Patient no.	No. of children	No. of daughters	No. of sons	Microchimerism
1	4	2	2	Yes
2	1	0	1	Yes
3	3	1	2	Yes
4	2	1	1	Yes
5	2	1	1	Yes
6	2	1	1	Yes
7	4	1	3	Yes
Mean	2.57	1	1.57	
9	1	0	1	No
10	2	1	1	No
11	1	0	1	No
12	1	0	1	No
13	0	0	0	No
14	0	0	0	No
Mean	0.83	0.17	0.67	
P value	0.009	0.013	0.035	

Patients with microchimerism have significantly more children (sons and daughters) than patients without microchimerism, whereas no differences were found between the latter patients and controls.

Adapted from Klintschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.

Mutations in *PTPN11*, Encoding the Protein Tyrosine Phosphatase SHP-2 Cause Noonan Syndrome

In approximately 50% of subjects with Noonan syndrome (NS is mapped to chromosome 12q24.1) the investigators identified mutations in the 15 exon gene (*PTPN11*) encoding the non-receptor protein [tyrosine phosphatase (PTP) - SHP-2]. This protein has two SH2 (Src homology docking) domains and a long enzymatic domain with the sites interacting to achieve an active or inactive state of function. Diverse missense mutations were found in the third exon encoding the amino-terminal SH2 (Src homology) domain and in three exons (7,8,13) encoding the PTP domain that apparently rendered the protein constitutively active. SHP-2 is a component of several intracellular signal transduction systems involved in embryonic development that modulate cell division, differentiation, and migration, including that mediated by the epidermal growth factor receptor. The latter pathway is important in the formation of the cardiac semilunar valves. The mutations associated with NS are in conserved amino acid sites in which the alteration leads to conformational changes that "lock" the protein in its enzymatically active state. The down-stream pathways that are affected by this "positive" change in enzyme activity have yet to be identified.

Tartaglia M, et al. *Nat Genet* 2001;29:465-468.

Editor's Comment: *Noonan syndrome (OMIM 163950) is characterized by "Turner-like" facial features, short stature, webbed neck, cubitus valgus, pulmonic stenosis (rather than coarctation of the aorta which is frequent in Turner syndrome), developmental delay, and bleeding diathesis. Since the Noonan phenotype is genetically heterogeneous, other genetic errors may exist, including mutations in the non-coding regions of PTPN11 that were not determined in the present report. The short stature and many of the skeletal abnormalities found in patients with Leri-Weill dyschondrosteosis and Turner syndrome (TS) have been attributed to haploinsufficiency of SHOX (chromosome Xpter-p22.32) either due to its deletion or to loss-of-function missense or nonsense mutations.*[1,2] *Given the visual similarity of the NS and TS phenotype, it will be of interest to determine if the proteins regulated by PTPN11 and SHOX interact. Might the product of SHOX be an inhibitor of SHP-2 generation or activity?*

Allen W. Root, MD

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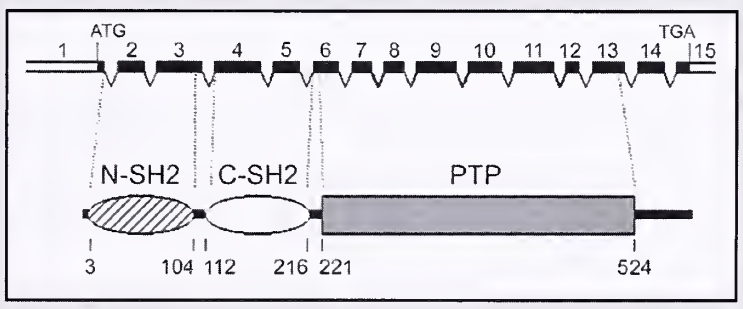
Figure



PTPN11 organization and SHP-2 domain structure. The coding exons are shown at the top as numbered filled boxes, and the positions of the ATG and TGA codons are indicated. The functional domains of the SHP-2 protein, comprising two tandemly arranged SH2 domains at the N terminus (N-SH2 and C-SH2) followed by a protein tyrosine phosphatase (PTP) domain, are shown below. Numbers below the domain structure indicate the amino-acid boundaries of those domains.

Reprinted with permission from Tartaglia M, et al. *Nat Genet* 2001;29:465-468.

Mothers with Congenital Adrenal Hyperplasia (CAH) and their Children: Outcome of Pregnancy, Birth and Childhood

The authors examined the gestational history of 122 women with 21-hydroxylase deficient CAH which was confirmed by genotyping in the majority. These women were born after 1948, followed in the investigators' clinic (University Children's Hospital, Munich) and were over 20 years of age at the time of study. Eighteen of the 122 women (15%) had delivered 31 children. The diagnosis of the 18 mothers was as follows: salt-losing, 1 of 48 total (2%); simple virilizing, 12 of 64 total (19%), and non-classical, 5 of 10 total (50%). The woman with salt-losing CAH had two miscarriages before delivering her child. One woman with non-classical CAH had two tubal pregnancies.

Conception occurred between 18-36 years (mean 28 years). The pregnancies were uneventful with the women receiving hydrocortisone, prednisone, prednisolone, or dexamethasone during gestation. Sixteen pregnancies required cesarean sections, primarily in women not having nonclassical CAH. Five of the 31 offspring were <10th percentile for gestational

age. One developed an intracerebral hemorrhage. An additional patient was microcephalic at birth. None of the 18 female offspring had malformation of the external genitalia. Follow-up of the 31 offspring, 6 of whom were less than 5 years of age, 7 of whom were between 5-10 years, and 18 who were older than 10 years of age at the time of evaluation, revealed that all were growing, maturing, and developing normally.

Krone N, et al. *Clin Endocrinol* 2001;55:523-529.

First Editor's Comment: *These data are encouraging in that women with simple virilizing and non-classical CAH are often able to conceive and deliver healthy children, thus confirming previous reports. More data on the degree of adrenal suppression during pregnancy, and knowing post-natal neonatal adrenal function would have been of interest.*

That only one of 48 women with salt-losing CAH had an infant illustrates the difficulties still encountered in the management of many of these patients. As Krone et al discuss, the relative infertility of women with CAH may be due to hormonal (hyperandrogenism), anatomic (inadequate reconstruction of the vagina), or psychosocial factors (behavioral masculinization, low marriage rate, and/or sexual preference). It is anticipated that prenatal detection and treatment of females with CAH and establishing neonatal screening programs for this disorder will change substantially the "natural history" of pregnancy in females with CAH.

Regarding surgical reconstruction of the external genitalia in the virilized female, while clitoroplasty may be appropriate in the neonatal period, vaginoplasty should be deferred until the peri menarchal period, as earlier reconstructive surgery is usually inadequate.[1] In 39 adolescent phenotypic females (20 with CAH) (mean age at examination 15 years) who underwent vaginal surgery in infancy at a median age of 10 months, Creighton et al found that approximately 60% had a good or satisfactory cosmetic appearance of the external genitalia, but almost all required further surgery to permit use of tampons during menses and, presumably, sexual relations in adulthood.

Allen W. Root, MD

Second Editor's Comment: *Much is being discussed and written in 2002 regarding surgery on the genitalia of patients with enlarged clitorises. The current recommendation of many surgeons and pediatric endocrinologists is that surgery on the clitoris be delayed in most cases in the newborn period. For more details the reader is referred to references 1, 2, and 3 below. A lead article concerning the dilemmas of gender assignment and surgery will be published soon in GGH to provide up-to-date considerations for you our reader.*

Robert M. Blizzard, MD

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Growth Hormone Improves Clinical Status in Prepubertal Children with Cystic Fibrosis: Results of a Randomized Controlled Trial

Hardin and colleagues studied the effects of recombinant GH (0.3 mg/kg/wk) in 10 children with cystic fibrosis (CF) (ages 7-12, Tanner stage I) as compared to a control group of 9 similar children. All children recruited for the study were $\leq 10^{th}$ percentile for both height and weight and had adequate caloric intake as determined on 2 evaluations. Only one had an abnormal growth hormone stimulation test. Children were excluded from the study if they had been hospitalized within 6 weeks or had been treated with systemic or oral steroids within 6 weeks. Evaluations were made of pulmonary functions including forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV_1). In addition, peak expiratory pressure (PEP) and peak inspiratory pressure (PIP) were measured. Resting energy expenditure, was determined using indirect calorimetry, and lean body mass was determined by whole body dual energy x-ray absorptiometry. Studies were made at baseline and every 3 months. Data were collected with regard to the number of hospitalizations and antibiotic therapy. All data for both the treatment group and the control group were similar at baseline.

The height and weight Z scores were significantly greater in the treatment group after one year than in the control group; furthermore the treatment group had a significant increase in lean body mass. Additionally, at 12 months the treatment group had a significant improvement in percent FVC, PIP, and PEP. There was no significant change in percent FEV_1. The GH treated group had a significant decrease in the number of hospitalizations, although outpatient antibiotic therapy was not different between the two groups. There was no significant change in resting energy expenditure or nutritional intake during the study and carbohydrate

intolerance did not develop in either group. The advancement in bone age over the 12 months was not different between the two groups.

The authors conclude that growth hormone therapy is of significant benefit to pre-pubertal children with CF in terms of their height, weight, body composition, pulmonary function, and number of hospitalizations.

Hardin DS, et al. *J Pediatr* 2001;139:636-642.

First Editor's Comment: *This study by Hardin and associates is the first randomized, controlled trial of growth hormone therapy in children with cystic fibrosis. The findings are highly significant, although they have only been collected for a single year. Many questions remain unresolved. It would be important for studies to be undertaken to determine whether or not the change in lean body mass was due to an improved use of ingested calories and protein as suggested by the authors. In addition, the long-term benefits of treatment need to be evaluated, and the optimal dose needs to be determined. Furthermore, it will be important to follow these children to determine whether or not they are at increased risk for glucose intolerance over time. Hardin and associates have provided the preliminary data necessary to undertake a much larger scale study of the use of growth hormone in these children.*

William L. Clarke, MD

Second Editor's Comment: *Growth Hormone treatment in patients with cystic fibrosis has been shown to be of benefit in various short-term trials.[1,2] However this is the first randomized controlled trial of GH treatment in patients with this disease. Growth hormone resulted in improved clinical status and increased growth. In CF, malnutrition develops as a result of unfavorable energy balance caused by a combination of poor intake, malabsorption of nutrients, chronic pulmonary disease and increased energy expenditures. Malnutrition adversely affects the course of the disease as well as the survival of the patients. Therefore any means to improve the anabolic state of CF patients may be of benefit. In this study GH treatment also improved the quality of life. Nonetheless, detrimental effects of GH treatment could occur in patients with CF, as diabetes is prevalent among this population.[3] Although in this study no patient developed this problem, the data cannot be extended to other patients or to those who would undergo a longer-term treatment. It should also be kept in mind that improvements in growth and nutrition status of CF patients may be accomplished with aggressive nutritional supplementation without GH treatment.[4]*

Fima Lifshitz, MD

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Intake of Vitamin D and Risk of Type I Diabetes: A Birth-Cohort Study

To ascertain whether vitamin D supplementation or vitamin D deficiency in infancy could affect the development of type I diabetes, a birth-cohort study was done in Oulu and Lapland, Finland. All infants born in 1996 were studied (n = 12,055). Data were collected on vitamin D supplementation and on the presence of suspected rickets during the first year of life. The primary outcome measured was the diagnosis of type I diabetes by the end of 1997 (30 year follow-up). Of the 10,366 children included in the analysis, 81 were diagnosed with type I diabetes. Vitamin D supplementation was associated with a decreased frequency of this disease. Children who took the recommended 2000 IU of vitamin D on a daily basis had a rate ratio of 0.22 of developing the disease, as compared with those who received no vitamin D. The rate ratio in those who received a lesser amount of vitamin D supplementation was 0.12. Children suspected of having rickets during the first year of life had a rate ratio of 3.0 as compared with those without such diagnosis. The authors concluded that vitamin D supplementation was associated with a reduced risk of type I diabetes.

Hypponen E, et al. *Lancet* 2001;358:1500-1503.

First Editor's Comments: *This is a very provocative study implicating the deficiency of one hormone (vitamin D) on the development of another hormone deficiency (insulin). The mechanisms of such association were thought to be related to the triggering of an immune process resulting from the lack of vitamin D. This is consistent with data from animal studies, and with the observation that cod liver oil supplementation during pregnancy is associated with a reduced rate of type I diabetes in the offspring.[1] The Eurodiab study also showed that vitamin D supplementation in early childhood may prevent this disease.[2] However, only 0.3% of infants in the Eurodiab study were not given*

vitamin D during the first year of life, thus the comparative population was rather small. The increased prevalence of this disease (3x) among children in this Finnish study, who were suspected of having rickets, is impressive. However the data are not very compelling since there was no radiologic or biochemical evidence of rickets presented.

The infants who took 2000 IU of vitamin D as a daily supplement had a 78% lower risk of developing diabetes. This dose of vitamin D, however, is high and not recommended by most authorities. (The Committee on Nutrition of the American Academy of Pediatrics, among others, state that an adequate intake of this vitamin is 200 IU per day.) Others have recommended dosages ranging from 400 to 1000u per day,[3] where there may be lack of sunlight exposure, particularly during the long winter months in the northern hemisphere. Although there is no single recommendation for the amount of vitamin D supplemented, exposure to the sun usually will satisfy the requirements to prevent rickets and vitamin D deficiency. As little as 1 minimal erythemal dose (MED) of sunlight is equivalent to ingesting about 10,000 IU of vitamin D. Simple exposure of hands and face two or three times per week provides a third to a half of the MED (about 5 minutes for fair-skinned people) is more than adequate. Moreover, sunlight is without risk of hypervitaminosis D which may occur when large amounts of vitamin D supplements are ingested. Thus, caution should be exercised to the possible temptation of increasing vitamin D supplementation in an attempt to prevent type I diabetes. Further studies are needed to assess if there are other factors to ascertain why there is a high prevalence of type I diabetes among populations who also are exposed to insufficient sunlight such as found in Finland.

Fima Lifshitz, MD

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Second Editor's Comment: *In the early 19th Century, cod liver oil was given to prevent rickets. The classical role of vitamin D in the prevention of rickets is to assist absorption of calcium and phosphate. Vitamin D also appears to play a role in preventing some cancers and autoimmune diseases. Ideally, in a study such as the one reported here, evaluation would include plasma 25(OH) D or 1,25(OH) $2D_3$ concentrations. When sun exposure is limited, as in northern Finland, supplementation or dietary intake is an important source of vitamin D. Breast milk does not contain enough vitamin D to cover an infant's needs. The role of vitamin D in the pathogenesis of type 1 diabetes certainly deserves follow-up. If vitamin D does impair the immune system functioning in infancy, there may be other long-term effects. Interesting as well, Finland has the highest incidence of type 1 diabetes in the world.*

Judith G. Hall, OC, MD

Beneficial Effects of Intensive Therapy of Diabetes during Adolescence: Outcomes after the Conclusion of the Diabetes Control and Complications Trial (DCCT)

The DCCT, in 1994, reported the results of intensive diabetes therapy of adolescents (age 13-17 years at the time of enrollment into the study). Those results demonstrated a significant reduction in the risk for the development, and progression of retinopathy and microalbuminuria. Since that time, subjects from both the intensive and conventional therapy groups have been offered the opportunity to participate in the epidemiologic study of diabetes interventions and complications (EDIC). EDIC is a long-term observational study of the DCCT cohort. In this manuscript the DCCT/EDIC research group presents their latest findings. Of the original 195 adolescents, 175 agreed to participate in the EDIC study. At the end of the DCCT all subjects returned to their health care providers in the community for continuing diabetes care, and all conventionally treated subjects were offered instruction in the use of intensive therapy. Approximately 50% of the subjects continued to receive their care at a DCCT/EDIC site. Subjects were seen on a yearly basis for determination of HbA1c and the recording of severe hypoglycemic episodes. Retinopathy was assessed by stereoscopic fundus photography at year 4, and classified according to the criteria described in the DCCT trial. A 3-step or more progression was classified as significant. Renal function was determined every other year by measurement of albumin excretion.

At year 4, 1/3 of the subjects who were originally randomized to conventional therapy continued to use 1 or 2 injections a day. The rest switched to multiple daily injections or insulin pump therapy. Ninety percent of former intensive therapy subjects continued to use multiple daily insulin injections or pump therapy. Total insulin doses and frequency of blood glucose monitoring

were similar between the 2 treatment groups. The difference in HbA1c between treatment groups was highly significant at the closeout of the DCCT, but by the end of the first year of the EDIC study there were no significant differences in HbA1c levels between the two groups. This was the result of both an increase in HbA1c by the intensive therapy group, and a decrease by the conventionally controlled group. These HbA1c values remained stable over the next 3 years (8.38% vs. 8.45%, intensive vs. conventional). In addition, the relative risk of severe hypoglycemia for patients in the former intensive treatment group was < 1, which was a decrease from the rates during the DCCT, and an increase in hypoglycemic occurrence for the conventionally controlled group. There was no difference in body weight, BMI, or percentage of subjects overweight at year 4 of the EDIC study.

After 4 years of follow-up in the EDIC study, 65% of the original conventionally treated patients showed a 3-step or more progression in retinopathy as compared with 32% of the former intensive group patients. This represents an odds ratio reduction of 74% for those having been in the intensive control group. Thus, the benefits of intensive therapy persisted for an additional 4 years in a significant number despite increased levels of glucose control. Similar findings were observed for the progression of nephrological disease. There was an 85% reduction in the adjusted odds ratio for developing albuminuria in the former intensive treated patients vs. the former conventionally controlled group. Thus, the benefits of previous intensive therapy continued for another 4 years with regard to renal function.

The authors state that these results demonstrate conclusively that the benefits of intensive therapy outweigh any associated risks of hypoglycemia and weight gain, and persist for at least four years. In addition, the data suggest that less than optimal glycemic control during the early years of diabetes (in adolescence) has a long lasting, detrimental effect on the development of complications even after better glycemic control is established. Thus the recommendation is that intensive therapy be the standard of care for adolescents with type I diabetes mellitus. The DCCT/EDIC study is planned to continue for at least 10 years.

The Diabetes Control and Complications Trial/Epidemiology of Diabetes of Interventions and Complications research group: *J Pediatr* 2001;139:804-812.

Editor's Comment: *The results of the DCCT/EDIC at year 4 in adolescents are not different from those presented for the entire group (New Engl J Med 2000;342:381-389). The findings are important and have significant implications for the treatment of adolescents starting at diagnosis, and perhaps pre-adolescent children with type I diabetes mellitus. Some have assumed that the intensive therapy achieved by the DCCT research group, while important in reducing complications, might not be a reasonable and cost-effective treatment regimen for all adolescents with diabetes. These data prove otherwise. Intensive therapy initiated early in the course of diabetes has prolonged and long-lasting effects of reducing the risks of microvascular complications. Alternatively, diabetes management resulting in poor glucose control during the early adolescent years may be associated with an increased risk of microvascular complications, even after intensive therapy and a reduction in HbA1c has been achieved. Thus, these data support the initiation of intensive diabetes therapy designed to achieve near normal glucose control as early as possible in newly diagnosed adolescents. This must be the standard of care. Patients, their parents, and third-party payers must be educated to understand, demand, and compensate for such treatment.*

William L. Clarke, MD

Growth in Human Immunodeficiency Virus Type 1-Infected Children Treated with Protease Inhibitors

About 33% of children infected with HIV have impaired growth. The extent of such impairment may be regarded as a clinical criterion predicting progression to AIDS. The addition of protease inhibitors (PIs) has been demonstrated to frequently reduce plasma HIV RNA levels, to increase CD4 lymphocyte numbers, and to improve the general condition of children and adults with HIV retrovirus type I infections.

Steiner et al present data on the long-term (72 week) impact of PI treatment on growth of infected children. Data are reported on 44 children between the ages of 0-17 years with confirmed infection. They were observed for 72 weeks prior to starting PI treatment. Nitonavir or nelfinavir were added to the previous treatment of two nucleoside analogue reverse transcriptase inhibitors. Growth, HIV-1 RNA plasma levels, and CD4 lymphocyte counts were determined at 0, 24, 48, and 72 weeks of treatment. Heights were reported in SD scores as determined from normal aged and gender individuals. Data from 44 children were analyzed in 3 age groups [6

children <3 years of age (group I), 23 children 3-10 years of age (group II), and 15 children >10 years of age (group III)]. All had completed 72 weeks of PI treatment. Multiple regression analyses were used to determine the relationship between parameters of growth and variables such as CD4 cell count and CDC HIV-1 categories. Children in group I were more frequently in the severe CDC clinical category "C" and had higher plasma HIV-1 RNA levels at baseline than those in groups II and III.

By 24 weeks of treatment, there was a significant decrease in mean plasma HIV-1 levels in children of group I vs. those in groups II and III. Twenty-seven of the 44 children showed a sustained reduction of HIV 1 RNA levels. In the 72 weeks before the initiation of PI therapy the differences between Δ-Z scores at 24 week intervals indicated progressive growth retardation which was reversed with a significant increase in growth during the 72 weeks after the PIs were added. This increase was biphasic with a greater increase between weeks 0-24, and a second increase between 48-72 weeks. The greatest increase in growth was in the 6 children in group I, all of whom had significant growth retardation at baseline and in the 4 significantly retarded children in group II. The 19 other children in group II and all 15 in group III had growth rates maintained within 1 SDS of the mean. Growth while receiving PIs was negatively correlated with growth during the preceding period, and positively correlated with an increase in CD4 cells. No correlation was seen between the decrease in plasma HIV-1 levels. Thus, age categories and CDC clinical categories were significantly associated with catch-up growth, but multiple regression analysis revealed that only growth during the preceding period and the age category were significantly associated with growth during PI therapy.

The authors note that previous studies have shown that stunting has been correlated with higher plasma HIV-1 RNA levels. Of note, the older children in the cohort were not as severely stunted as the younger children, and did not have as significant a growth response to PI therapy. The authors speculate that these findings may be the result of the older children having a slower progression of HIV infection than the younger children, since they survived infancy in the era prior to aggressive therapy. In addition, the authors point out that others have attributed stunting in HIV infected children to sub-clinical hypothyroidism, low IGF-1, or proteolysis of IGF BP3. The authors did not measure these hormone levels.

Steiner F, et al. *Eur J Pediatr* 2001;160: 611-616.

Editor's Comment: *The data reported in this paper by Steiner, et al are important from two aspects. First, treatment with a protease inhibitor can improve growth rates in young HIV infected children. Secondly, those with the greatest catch-up growth are those who are the most stunted initially. Such information is similar to that which has been shown for treatment of nearly every chronic disease of childhood. Unfortunately the authors did not determine biochemical markers of growth, including IGF-1 and IGF BP3. They suggest this be done in future studies. These data might have been useful in helping decide which children could benefit the most from such therapy. The data presented, however, are clinically useful.*

William L. Clarke, MD

Paternal Contribution to Aneuploidy

The relationship of maternal age to chromosomal abnormalities is well established; however, there have been conflicting data with regard to paternal contribution. Of potential pertinence is that 10 – 30% of autosomal trisomies arise during paternal meiosis, 100% of XYYs and 50% of XXYs are paternal in origin, and 80% of Turner syndrome patients are missing the paternal X. Also, an increase in paternal age is associated with the development of uniparental disomy 15, and trisomy 18 is seen with increased paternal age. To further study the relationship of paternal age to diploidy and disomy of sperm, the authors of this paper screened human sperm using four-colour FISH probes. Chromosomes 6, 21, X, and Y were examined to determine the incidence of disomy in sperm related to paternal age where the normal usual sperm are haploid.

Almost 200,000 sperm were examined from 18 healthy donors, ages 24 to 74. The investigators found a significant increase in the level of autosomal disomy and a marginally significant increase in sex chromosome disomy with increasing male age. Significant individual variation was observed. The increase in disomy ranged from 0.3 to 17% for each 10-year period. This suggests that older men have a tendency to show synaptic abnormalities perhaps related to the deterioration of testicular environment with advancing age.

Bosch M, et al. *Euro J Hum Gen* 2001;9:533-538.

Editor's Comment: *There is a growing interest in paternal contributions to congenital anomalies, both potential teratogens and the effect of aging itself. Although triploids are not usually viable, it is interesting*

that paternal age would seem to lead to an increased contribution to triploid conceptions. This could also play some role in triploid-diploid mixaploid individuals. This article is an excellent review of current knowledge pertaining to diploidy, aneuploidy, and disomy in the sperm of males of various ages and in various chromosomally determined clinical conditions.

Judith G. Hall, OC, MD

New Syndrome of Hyperinsulinism and Hyperammonemia

Although there are many causes of hypoglycemia, a new syndrome associating hyperinsulinism with hyperammonemia was recently described (Zammarachi, et al. *Metabolism* 1996:45:957; Weinzimer, et al. *J Pediatr* 1997:130:661; Stanley, et al. *N Eng J Med* 1998:338:1352). This syndrome is identical or closely related to the leucine-sensitive hypoglycemia syndrome and is congenital in origin. Clinical manifestations are usually observed in neonates and/or infants. The diagnosis of patients with HSS is crucial as therapy differs radically, medical and not surgical, from that of other hyperinsulinemic patients. A positive response to diazoxide- and/or leucine-free diet is usually observed. All but one of the 12 patients in the article by De Lonlay had at least a partial response to diazoxide.

Genetically all 12 cases studied seem to be new mutations, as they occurred sporadically without family histories. This mutation results in a gain of function in the glutamate dehydrogenase gene (GLUD1). It also results in a decreased inhibitory effect of guanosine triphosphate on the enzyme. It has been suggested that the elevated oxidation of glutamate to á-ketoglutarate stimulates insulin secretion by increasing the ATP/ADP ratio in the pancreatic Beta cell, although this is unproven. All 12 patients studied had mutations located within or outside the GTP binding site, without any correlation between phenotype and genotype. The mutations in the GLUD1 gene are found in exons 6, 10, 11, and 12, which includes the antenna region of the enzyme and the GDP binding domain.

In a review of hyperinsulinemic patients by the authors in their institution over the past 20 years, plasma ammonia concentrations were measured in 71 (45 neonates and 26 infants) and hyperammonemia was found in 12 of the 71. The incidence of this type of hypoglycemia is significant. The authors conclude that ammonia concentrations should be measured in every patient investigated for hyperinsulinism and that, conversely, hypoglycemia should be looked for in all patients with unexplained hyperammonemia.

De Lonlay P, et al. *Pediatr Res* 2001;50:353-357.

Editor's Comment: *Heterogeneity is the name of the game, and molecular techniques allow us to recognize many of the reasons for heterogeneity. Within heterogeneity, many new biochemical pathways and mechanisms of disease are being identified. As in the case of this syndrome, different types of therapy become most appropriate.*

Judith G. Hall, OC, MD

15 Years After Chernobyl: New Evidence of Thyroid Cancer

A striking increase in childhood thyroid cancer was reported after the Chernobyl accident. Because proper dosimetry was not done at the time, the exact amount of exposure to children was not clear. The children who attended school within a 150 km radius of Chernobyl have been carefully screened over the ensuing 14 years. The nuclear power plant accident happened on April 26, 1986. One case of thyroid cancer was recorded per 2,409 children born between April 27, 1986 and December 31, 1986, (intrauterine exposure). A much higher rate, with 31 thyroid cancers among 9,720 children (ages 1 day – 4 years), was seen in those born in the 4 years prior to the accident. Over 20,000 children have been followed and repeatedly examined using ultrasound, as well as measurements of TSH, free thyroxine and thyroid peroxidase antibodies. An increase in thyroid cancer has not been seen in children born since 1987 (post Chernobyl conceived). All of the cancers were papillary adenocarcinomas.

Shibata Y, et al. *Lancet* 2001;358:1965-1966.

Editor's Comment: *The conclusion of this follow-up study is that children at a young age and probably up until 10 years of age are at particularly high risk for developing thyroid cancer after exposure to radioactive fallout. Hopefully, there will never be another Chernobyl. If there is, careful dosimetry to know the amount of exposure and the rapidity of decay will be important. However, it is clear that children, particularly young children, are at the greatest risk and need to be followed carefully.*

Judith G. Hall, OC, MD

Letter to the Editor

I read the commentaries and the review of the paper by Zucchini et al on SCA final height after growth hormone treatment from *Arch Dis Child*, in the October 2001 issue of *GGH*. I would like to add the following points.

As the reviewer states, the treatment had begun late (approximately 10.8 years). What the reviewer does not state clearly is that the GH dose was too low. I calculated the dose to be about 0.22 mg/kg/week. The FDA approved GH for SGA at a recommended dose of 0.48 mg/kg/week. It is not surprising therefore that less than 50% of the recommended dose gives disappointing results. de Zegher et al presented near final height at the joint meeting in Montreal and the robust height SDS gains appeared to be sustained.

In essence then, the disappointing results of the Zucchini paper can be summarized as "too little, too late". That conclusion did not come across in the comments.

Paul Saenger, MD

First Editor's Comments: *We appreciated the remarks of Dr. Saenger with regard to the abstract of the article by Zucchini,etc, Arch Dis Child 2001 84:340. Although, as Dr. Saenger pointed out, the dosage of GH used in the study was significantly less than that approved by the FDA for treating short SGA children, the children treated in this study were classified as growth hormone deficient based on stimulation tests. Thus one might argue that the magnitude of the difference between recommended and actual GH dose was not as different for these GH deficient children as it might have been had they been GH sufficient. Indeed, the presentation by de Zegher in Montreal last summer was very encouraging. Long-term studies, treating SGA children from an early age, at the recommended dose are necessary to answer the question of the overall benefit on adult height of GH treatment of SGA children.*

William L. Clarke, MD

Second Editor's Comment: *The Reviewer thanks Dr. Saenger for his helpful comments about the manuscript of Zucchini et al[1] concerning the effect of rhGH in short children born small for gestational age (SGA). The dose of rhGH utilized by these investigators (calculated to be 0.27 mg/kg/week) was indeed less than that employed by de Zegher et al[2,3] (ranging between 0.23 and 0.7 mg/kg/week). In addition, these investigators began treatment with rhGH between 2-8 years of age, thus affording longer treatment periods. The adult heights of their patients have not been reported as yet, although through 6 years of therapy there was an increase in height of +2 SDS. However, treatment with high doses of rhGH resulted in insulin resistance that may not be completely reversible[4] and in high levels of IGF-I during treatment.[5]*

Even if rhGH is able to increase adult stature to a statistically significant extent, there are no data indicating that greater height is meaningful in terms of improved psychosocial well-being, educational attainment, or economic success. Given the potential hazards of insulin resistance, elevated levels of IGF-I (if only temporary), and lack of documented enhancement in the quality of life (QoL), treatment of SCA children with rhGH, particularly at the dose that has been approved by the FDA, seems hazardous to this reviewer and should only be employed in an investigative setting until its statural and QoL efficacies and safety have been well documented.

Allen W. Root, MD

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2. de Zegher F, et al. *J Clin Endocrinol Metab* 2000;85:2816-2921.
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Phallic Construction 2002: Current Concepts and Future Directions

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INTRODUCTION

The penis is anatomically complex, being involved with both voiding and sexual activity. Both have significant psychosexual implications for affected patients. While the functions of sperm and urine transport may be bypassed using modern technology; we are as yet unable to replicate the unique anatomic and biomechanical properties of the penis. Therefore, current attempts at replacement of an absent or inadequate penis are designed to create an acceptable phallus or penis-like structure. These reconstructive efforts are referred to as phallic construction or phalloplasty.

The optimal phallus should provide all of the following: 1) both tactile and erogenous sensibility, 2) a neourethra which allows voiding while standing, 3) the capability to permit prosthetic insertion which permits successful vaginal intromission, 4) cosmetically aesthetic acceptability of both the phallus and proposed donor sites, and 5) acceptable phallic growth to adult size in the case of pediatric phalloplasty. Optimally the surgery should be accomplished in a reproducible single stage with acceptable morbidity. Modern reconstructive and microsurgical techniques permit us to achieve these aims much of the time. However, single stage reconstruction eludes us in most cases.

Phallic construction is one of the most challenging procedures in reconstructive surgery. At our center we use a multi-disciplinary approach which includes urologists, plastic surgeons, gynecologists, endocrinologists and other experts. The purpose of this review is to discuss the history of phallic construction that has led to current techniques of phalloplasty. These will be briefly outlined in order to address some of the most recent indications for phallic construction, which include the procedure's use in trauma patients, in patients with congenital anomalies, and in transgender patients. Discussed are our results in each patient subcategory.

HISTORY

The evolution of phallic construction techniques has paralleled advances in reconstructive surgery. Initially, random tubed skin flaps were used, which were transferred in tubed delay fashion. These techniques were supplanted by the use of island and/or musculocutaneous flaps. With the advent of modern microsurgical techniques, microvascular free transfer flaps have become the state-of-the-art for phallic construction.[1]

Bogoraz[2] reported the first successful phallic construction in 1936. He employed an abdominal tubed flap to construct a phallus, in a case of post-traumatic penile amputation. This patient ultimately had successful intercourse using a segment of rib cartilage implanted into the phallus as a stiffener, and fathered children after the reconstruction.

Maltz[3] and Gillies and Harrison[4] are credited with developing the tube within a tube concept which permits a second inner tube to function as a urethra within the outer phallic shaft. Because the urethra was fashioned from hair-bearing abdominal midline skin, urethral

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strictures and fistulas were the rule. Also the unreliable blood supply of the lower abdomen often compromised the flap's overall viability. Despite its aesthetic and functional limitations, variations of this abdominal flap remained popular throughout the 1950s and 1960s. In some cases, the inner tube was used for baculum placement to induce rigidity and not for voiding function.

A major step forward in phallic construction was achieved when Noe et al[5] used the reliable abdominal branch of the external pudendal artery to vascularize the phallus. Using more reliable vascularity, musculocutaneous flaps were successfully constructed by Orticochea,[6] Horton et al,[7] and others. Although these flaps were more aesthetically pleasing and more reliable, they remained insensate, and often required multiple "touch up" surgeries to achieve an acceptable result.

Puckett and Montie[8] performed the first microvascular free transfer flap phalloplasty in 1978. The seminal work of Gilbert et al[9] provided erogenous sensation to the phallus via anastomosis of a sensory nerve within the flap to the patient's pudendal nerve and the radial forearm flap single stage phalloplasty described in 1984 by Chang and Hwang[10] brought this evolving field to the current position. Additionally, Lovie et al[11,12] described the use of the ulnar forearm flap for head and neck reconstruction and Gilbert et al[13] used this flap for phallic construction, which became this center's procedure of choice.

SURGICAL ADVANCEMENTS

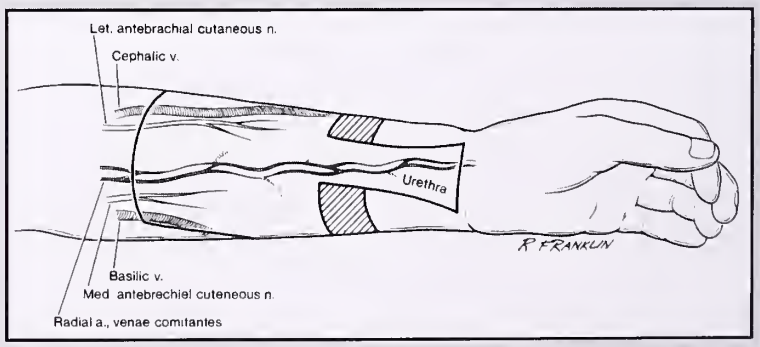
The free forearm flap is the gold standard for the modern phallic construction. These flaps are ideal from a technical standpoint, as they are malleable, and they remain relatively hairless, thus improving the aesthetic result. All of the currently employed forearm flap designs share certain common features, including arterial inflow from either the radial or ulnar artery (and venous drainage via basilic, cephalic veins and/or vena comitans), and erogenous sensation provided by either the medial or/and lateral antebrachial cutaneous nerves (Figure 1). A drawback to this flap is the post-operative appearance of the donor site. While functional or sensory problems are rare to non-existent within the forearm or hand, the cosmetic appearance may be disturbing to some patients. The appearance of this site can be improved by resurfacing the forearm with a full thickness skin graft from the groin. Other phallic construction options have been employed in patients who refuse forearm scars including fibula osseocutaneous flaps[14,15] and metaidoioplasty (plastic surgery to convert a clitoris to a penis),[16] but these are, we feel, clearly sub-optimal choices.

The original Chang & Hwang flap centered the phallic shaft around the radial artery, with the neo-urethra somewhat distant to the principal blood supply. The Biemer modification of this design centers the neo-urethra over the central portion of the flap, with the phallic shaft created by two skin islands separated from the neo-urethra by de-epithelialized strips. This modification results in less ischemic injury in the area of the neo-urethra, and allows for extension of the neo-urethra both proximally and distally along the length of the shaft. This extra length may be critical for a reliable anastomosis to an often foreshortened native urethra. The main disadvantages of this modification, when based on the radial artery, are that the urethra is centered over the hairiest portion of the forearm and two suture lines result from closure of the skin island around the neo-urethra.

Classically, the forearm flap was based upon the radial artery but in our hands it is based upon the ulnar artery,[12] since the increased caliber and length of the ulnar artery makes the anastomosis of the vascular pedicle technically more straight forward. Furthermore, the relatively hairless skin overlying the ulnar aspect of the forearm usually is best suited for urethral and phallic construction. Over the last 10 years, this center has adopted the ulnar forearm flap which also provides for construction of an integral neoglans (Figure 2).

Preoperative evaluation focuses upon the patient's general health, particularly from a cardiovascular standpoint. Heavy smoking with its associated vascular disease is an absolute contraindication to this type of microsurgery. The vascularity of the non-dominant forearm is assessed with the Allen test, followed by selective upper extremity Doppler sonography or angiography as needed. To date, we have not had upper extremity complications related to diversion of the ulnar arterial blood flow.

Figure 1



This view of the modification of the forearm flap (Farrow and Boyd) demonstrates the relationship of the neourethra to the nerves and vessels (radial artery) preserved for tactile sensation and organ viability respectively.

Reproduced with permission from WB Saunders, Campbells Urol. 7th Edit. 3:1998.

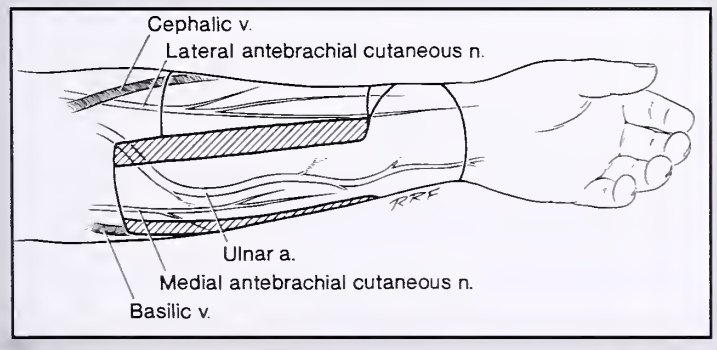
The flap is carefully designed with dimensions specific to the patient's requirements for phallic and urethral length. Dissection is carried out superficial to the deep antebrachial fascia, allowing for an extra tissue layer overlying the nerves and muscle tendons of the forearm. The ulnar artery, basilic and cephalic veins, and medial and lateral antebrachial cutaneous nerves are each meticulously dissected through the forearm and elevated with the flap. After the flap has been elevated, it is tubularized while still perfused on the forearm. The central skin island (neo-urethra) is tubularized, after which the outer phallic islands are tubularized. Finally, the newly constructed glans is transposed over the distal shaft.

The phallus is transferred to the anatomic area of the penis. The ulnar artery is typically anastomosed to the deep inferior epigastric artery, and the veins are anastomosed to either the deep inferior epigastric vena commitans, or to the saphenous veins. The urethral anastomosis is performed after vascularity has been restored. The sensory nerves of the flap are coapted to the dorsal nerves of the penis or clitoris; or in some cases, to the deep internal pudendal nerve. At the end of the procedure, the patient has a natural appearing phallus (Figure 3), and this appearance is further enhanced by scar remodeling in the subsequent year. The final step is forearm donor site coverage with thick full-thickness skin grafts – usually harvested from the groins.

SEXUAL FUNCTION OF THE NEOPHALLUS

The goal of achieving reliable phallic rigidity has remained a challenge in the field of phallic construction. Many options have been attempted with variable results. Occasionally, the neophallus may possess enough intrinsic stiffness to allow intromission without a prosthetic stiffening device. The original technique of Bogoraz[2] involved implantation of rib cartilage in the phallus, and for several years thereafter cartilage or nonvascularized bone were the standard approaches to obtaining phallic rigidity. The disadvantages of these techniques included warping and resorption of the cartilage/bone with time. Others[17] used vascularized bone segments incorporated in the phallus to provide rigidity. Another option has been to create a separate tube for a removable baculum.[17]

Figure 2



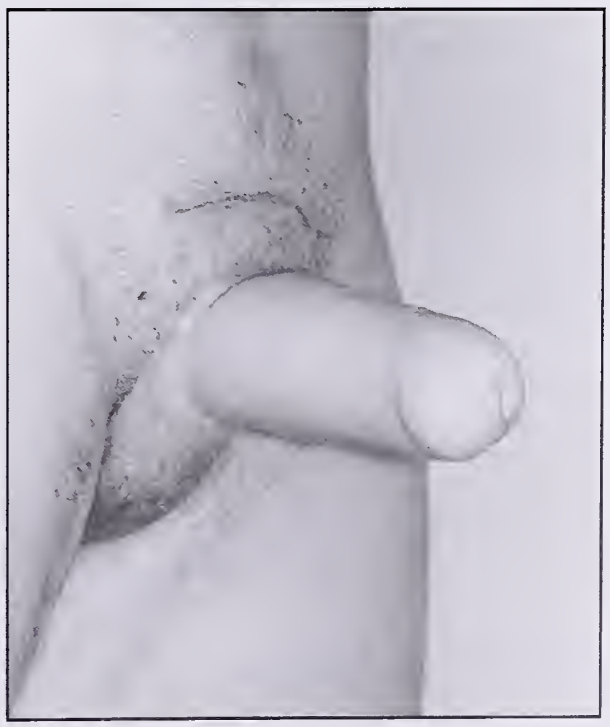
The forearm flap utilizing the ulnar artery as the source of blood supply and the antebrachial cutaneous nerves for tactile sensitivity are show in this diagram.

Reproduced with permission from WB Saunders, Ehrlich/Alter Reconstructive and Plastic Surgery of the Genitalia: Adult and Pediatric. 1999.

Prosthetic implants also have been inserted successfully.[18] The phallus usually develops tactile sensitivity between 4 and 9 months postoperatively. Such sensitivity must be present to protect against pressure necrosis prior to implanting a prosthesis. Also, the neourethra must have proven to be durable and infection free by this point. Unlike patients who have suffered traumatic penile amputation, congenital aphallic patients and female to male transgender patients lack corporal bodies in which to seat and anchor the prosthetic device to the pelvis.

In order to circumvent this problem, we have created the "neotunica," which is a Gore-Tex (polytetrafluoroethylene) graft, which acts as a sleeve surrounding the actual implant.[18,19] In a transgender patient without corporal remnants, the cylinder is ensheathed in the Gore-Tex sleeve, and the sleeve is then anchored to the periosteum of the ischial tuberosity (inferior pubic ramus) as well as to the pubic symphysis. If a hydraulic prosthesis is used, the pump is placed in the scrotum. If corporal remnants are present proximally,

Figure 3



The phalloplasty should, and often does, result in a favorable cosmetic result. Rigidity may need to be enhanced by utilizing one of the available prosthesis (see text).

they may be opened and used to seat the cylinders. The neotunica is then used to surround the distal ends of the prosthesis.

The category of prosthesis used is partially dependent on patient preference. Articulated as well as hydraulic implants have been employed. At this center we have had good results with the Duraphase® prosthesis and the AMS 700CX® prosthesis. Early in our experience, we tended to place single "rods," however we now place dual "rods" in the majority of cases. Two rods provide better rigidity, and are felt to have less potential for erosion.

INDICATIONS FOR PHALLIC CONSTRUCTION

Trauma

Penile amputation injuries have devastating psychological consequences that usually persist throughout the victim's lifetime. In North America, these injuries are fortunately rare. If the patient presents with the amputated tip of his penis, replantation offers excellent results and can be reviewed further.[19] In many cases the patient does not present with the severed part, and other reconstructive options – including phallic construction– must be entertained.

Pediatric Phallic Construction

Phallic construction in the prepubertal population continues to be a controversial topic, but should be considered for two broad categories of children. The first and less controversial category consists of boys who have sustained trauma to the penis. These boys have already been assigned the male gender. These patients usually are not candidates for gender reassignment, and phallic construction permits these boys to maintain their male gender identity.

The second category of patients who may be considered, consists of genetic XY babies who have a congenitally anomalous penis and often genital ambiguity. These babies may have classic micropenis, aphallia, partial androgen insensitivity or an enzymatic defect such as 5 alpha reductase deficiency. Also boys with cloacal exstrophy may fall into this category, although cloacal exstrophy is not classified as an intersex condition. While boys with classic exstrophy/ epispadias complex are typically able to function after epispadias repair and chordee correction, the rare patient may have corporal bodies that are inadequate to reconstruct even the most rudimentary penis. Phallic construction has been successful in some of these patients.[20]

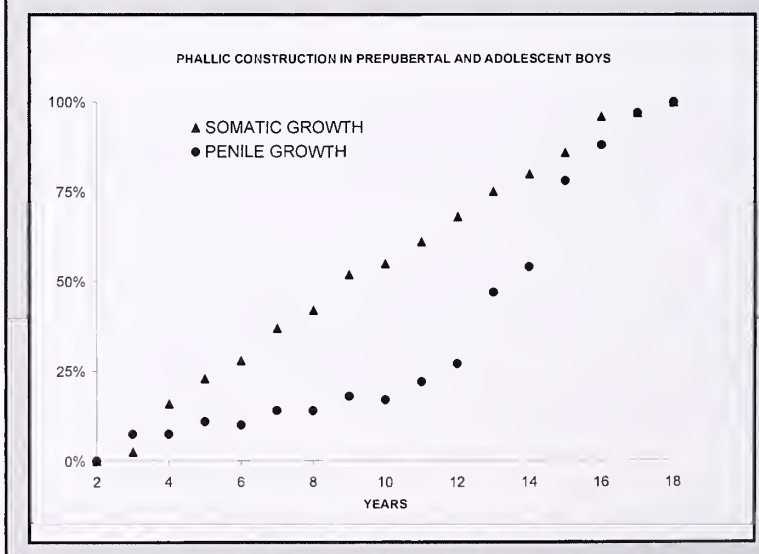
In prior years, many of the patients with micropenis, aphallia and exstrophy were gender-converted in early childhood and reared as girls. The fact that many such patients have experienced gender dysphoria later attests to the validity of the hypothesis that the genetically male brain is "masculinized" in utero. The advent and success of modern phallic construction techniques now permits these males to retain their genetic sex, and in rare patients potentially procreate later in life.

The timing of pediatric phallic construction remains of paramount importance. The key issue is construction of a phallus which is of appropriate size for a child, but which will reach adult dimensions post-pubertally. The normal penis is an androgen sensitive organ which grows to adult size during puberty under the influence of dihydrotestosterone. A forearm flap phallus is not androgen sensitive, and will grow at the rate of other somatic tissues. Therefore the somatic and genital growth rate must be factored into the equation when calculating relative flap size at any age (Figure 4).[21] We recommend construction of the neophallus between the ages of 6 and 8 years of age for patients in the pediatric subgroup.

Female-To-Male Transsexualism

Gender dysphoria is a widely recognized psychological condition wherein the patient is of normal phenotype but feels "trapped" in the body of the wrong sex. The incidence of this condition in the United States is approximately 1:50,000, with a male:female ratio of approximately 6-8:1.[22] Most psychiatrists believe that conversion of adult transsexual patients via psychotherapy back to their biologic sex is nearly impossible. Many of these patients benefit from hormonal and surgical gender reassignment.

Figure 4



Penile growth normally has an adolescent growth spurt. Phalluses constructed from forearm flaps have growth more in accord with somatic growth although of more limited nature.

Reproduced with permission from David Gilbert, previously unpublished.

Transgender surgery should be performed only at centers devoted to the complete care of these patients, as psychologic and medical needs require integrated assistance.

Our center utilizes a multi-disciplinary approach to these patients, utilizing the combined skills of two clinical psychologists, a gynecologist, two urologists and a plastic surgeon. Patients are evaluated by all members of the committee before acceptance for transgender surgery. (The Harry Benjamin criteria).[23]

Transsexual patients qualifying for phallic construction at our center undergo surgery in multiple stages. The first stage consists of hysterectomy and oophorectomy (usually via a vaginal approach), vaginectomy, colpocleisis, and urethral lengthening. The second stage, phallic construction, is as already discussed. Prosthetic placement is done at a third stage in those select patients that request it.

RESULTS OF PHALLIC CONSTRUCTION

Between 1986 and 1994, 40 patients underwent phallic construction at this center. Another 34 patients have undergone phallic construction between 1994 and 2001. Of the first 40 patients, 22 were female to male transgender patients. As previously mentioned, the introduction of the staged approach, with urethral lengthening, has reduced the incidence of difficult fistulas in this group. Thirty-four of 40 patients were available for follow-up. Stricture at the neourethral anastomotic site occurred in 68%, and urethrocutaneous fistulas at the penoscrotal junction in 32%. At the time of that review in 1993,[24,25] 68% of the series were symptom free or required only self-dilation. The modification of staged reconstruction, along with anastamotic covering with a muscle or fascial flap has reduced the overall urethral complication rate to about 30%.[25]

The results of recent penile prosthesis implantation have been more encouraging than previously reported by this center and others. We reported 8 patients in whom prosthetic implantation was attempted, 6 (75%) still have prostheses in place.[26] Infection necessitated prosthesis removal in 4 patients, of whom two were successfully reimplanted. Seven of 8 patients have been sexually active using their prostheses. The infection rate has declined in the past several years secondary to the introduction of perioperative closed suction drains and broad spectrum antibiotics. We currently have reported approximately 40 patients with only 2 explants in the last 20 patients.[27] One was performed for delayed erosion, and the second for vascular compromise of the flap in the immediate post-implant timeframe.

We have performed phallic construction in the pediatric population,[21] in 7 prepubertal and 4 adolescent boys, as well as in 5 older boys who had reached 18-24 years of age. Only one flap failed in the childhood/adolescent group (91% success rate). All patients who underwent flap nerve coaptation to the pudendal nerve reported return of protective sensation. All of the adolescents/young adults who underwent phallic construction noted erogenous sensation and the ability to orgasm. The question of flap growth in the pediatric subgroup is currently under review.

CONCLUSION

While phallic construction remains a challenging aspect of reconstructive surgery, it has evolved tremendously since its inauspicious beginnings in Russia in the 1930s. Modern phalluses are mostly aesthetically acceptable, durable, and in many cases very satisfying for the patient. Urethral reconstruction in the neophallus also has improved considerably, with reduction in the number of recorded fistulas and strictures. The search for an autogenous tissue source to facilitate rigidity continues, but we and others have had significant success thus far with the use of prosthetics in carefully selected patients. The prepubertal phallic construction continues to stir debate; but we believe that for genetic males, it presents an alternative to gender conversion, and patients must be so counseled.

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Future Articles

The Current Frontiers of In Vitro Fertilization

Molecular Genetics of Peripheral Precocious Puberty

Controversies in the Treatment of Intersex

Agonadia, Germ Cell Failure & Other Multiple Malformation Syndromes Associated with Gonadal Failure

SOMATOMEDIN HYPOTHESIS: TIME FOR REEXAMINATION

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The following article is a slightly modified article from *The Endocrinologist* 2001:470-473 and is reproduced by permission. The editors suggest you read The Letter to the Editor on page 48 before proceeding.

In 1957, Salmon and Daughaday[1] observed that incorporation of radioactive precursors of cartilage acid mucopolysaccharides could be stimulated in vitro by serum from hypophysectomized rats that had received growth hormone (GH) in vivo. Addition of GH directly to the medium, however, did not enhance precursor incorporation. The authors inferred that GH did not act directly on cartilage; instead, it did so by generation of a factor in the serum that enhanced the incorporation. The serum factor was originally named "sulfation factor", because radioactive sulfate was used as the precursor. The magnitude of the effect was proportional to the volume of serum used, and the factor was originally used as a bioassay for GH activity.[2] The in vitro incorporation test was discarded when radioimmunoassays of GH became available.[3] Subsequently, the sulfation factor was renamed "somatomedin", because it seemed to be the effector by which GH stimulated somatic growth.[4] Several somatomedins were identified, and the components of the system were designated by letters of the alphabet, as somatomedin A, B, and C.[5]

Before the development of a radioimmunoassay for insulin,[6] its activity in serum was measured by bioassay of its effects, such as glucose uptake by isolated tissues in vitro.[7] Radioimmunoassays of serum from fasting animals, however, showed that as little as 10% of the effect on serum glucose was caused by insulin itself.[8] Furthermore, the insulin-like activities were minimally suppressed by the addition of anti-insulin antibodies to the serum.[9] The "noninsulin" effects were attributed to the presence in the serum of nonsuppressible insulin-like activities, and a nomenclature was subsequently adopted designating them as insulin-like growth factors (IGF).[10]

The amino acid sequences of two nonsuppressible insulin-like activities (IGF-1 and IGF-2) were elucidated by Rinderknecht and Humbel,[11] and their tertiary structures were subsequently determined by Blundell et al.[12] They consist of A-domains homologous to the A-chain of insulin, B-domains homologous to the B-chain, C-domains homologous to the C-chain of proinsulin, and D-domains that extend from the C-terminals of the A-chains. Analysis of somatomedin-C, the principal growth factor of the somatomedin family, showed that it had the same amino acid sequence as IGF-1, and the two were considered to be identical.[13] Because the largest fraction of IGF-1 in the circulation is derived from the liver, where the expression of the gene is regulated by GH,[14] the *somatomedin hypothesis* was developed. It stated that the anabolic effects of GH on cartilage and other tissues were mediated through IGF-1 synthesized in the liver and not by direct action of GH on these original target tissues.[4] Although the hypothesis has gained widespread acceptance, there is mounting evidence that it may have to be modified or even abandoned. A priori, it would seem unlikely that a factor that *exerts* hypoglycemic effects[15] should be the effector of GH action.[16] Since GH is an insulin counter-regulatory hormone,[17] it seems paradoxical that it should exert its effects through a factor that produces hypoglycemia.

Isaksson et al[18] summarized evidence available in 1985 that GH acts directly on prechondrocytes, epiphysial plate cartilage, cloned preadipose cells, and myoblasts without the intervention of a mediating factor. GH also has been found to act directly on other tissues in vitro, such as stimulating erythropoiesis in vitro.[19]

More recently, additional evidence doubting the somatomedin hypothesis has accumulated. The evidence comes from three different sources. First, Salmon and Burkhalter[20] revisited the experiments originally conducted by Salmon and Daughaday[1] that formed the basis for the hypothesis. In these newer studies, they found that in contrast to their earlier experiments, GH added directly to cartilage from hypophysectomized rats did stimulate incorporation of radioactive sulfate into proteoglycans and radioactive

thymidine into DNA. They ascribed their newer findings to the use of a different medium in the more recent experiments; HEPES-buffered amino acid-glucose solution with a low concentration of bovine serum albumin. Amino acids were not added to the medium used in the original experiments, and the authors also speculate that a nondialyzable component of hypophysectomized rat serum may have inhibited the incorporation of sulfate into cartilage.

Secondly, a series of observations that cast doubt on the hypothesis was reported by Yakar et al[21] who devised an elegant set of experiments to determine if hepatically derived IGF-1 is the circulating mediator of GH effects on postnatal growth and development. Using the Cre/*lox*P recombination system, they deleted the IGF-1 gene exclusively in the livers of mice. Their finding of a 75% reduction in the concentrations of IGF-1 in the serum confirmed that the liver is the primary source of circulating IGF-1. Despite this reduction in circulating IGF-1, there was no evidence of growth impairment when the liver IGF-1-deficient mice were compared with their wild-type litter mates. These experiments have been confirmed by Sjogren et at[22] using the model devised by Yakar et al.[21]

A third observation casts doubt on the hypothesis. This concerns the issue of the lipogenic properties of IGF-1. In a report of long-term treatment of European patients with GH insensitivity syndrome, IGF-1 treatment led to accelerated growth, but there was also a substantial gain in fat mass that correlated significantly with the increase in height.[23] Ecuadorian patients with the same syndrome experienced a significant increase in growth rate when treated with IGF-1.[24] They also experienced a relative increase in mean body weight for height when they were treated with the higher of two doses of IGF-1.[24] It should be noted that not all investigators have reported an increase in fat mass.[25] Increased lipogenesis has also been shown to occur in a subject with an IGF-1 deletion who was treated with IGF-1.[26] The authors inferred that the lipogenic effects could be ascribed to the reduced concentrations of GH in the serum after IGF-1 treatment. This explanation is untenable, however, because increased lipogenesis was also found in the subjects with GH insensitivity syndrome.[23,24]

Increased fat mass is inconsistent with the hypothesis that IGF-1 mediates the effects of GH, which is a lipolytic and anabolic hormone.[27] It is more in keeping with an insulin-like action, such as that seen in infants of mothers with diabetes in whom hypoglycemia is prevented by placental exchange of glucose despite high concentrations of insulin in the fetal circulation.[28] The increased length and fat content of these infants is evidently because of the anabolic and lipogenic effects of insulin secreted by the fetal pancreas.[29,30] In considering the role of IGF-1 in growth promotion, distinguishing between the effects of circulating IGF-1 and IGF-1 produced by autocrine/paracrine mechanisms is important. In their experiments, Yakar et al[21] found that growth was severely restricted in IGF-1 knockout mice in which the gene was deleted from all tissues. There can be little doubt, therefore, that the IGF and their binding proteins are important growth factors when produced locally by autocrine/paracrine mechanisms. Moreover, as pointed out previously, expression of the hepatic gene for IGF-1 is regulated by GH,[14] and plasma concentrations of IGF-1 are uniformly increased in adults with acromegaly and children with gigantism.[31] Despite earlier findings that plasma IGF-1 and IGF-binding protein-3 concentrations might be useful in the diagnosis of GH deficiency, there are substantial disagreements on this issue.[32-34]

It is time to take note of the deficiencies in the hypothesis and possibly to abandon it completely. There is a strong body of evidence that liver-generated IGF-1 is unlikely to be responsible for the linear growth effects of GH and that the actions of GH on its target tissues do not require mediation by this factor in the circulation. It is also unlikely that measurement of these growth factors and their binding proteins in the plasma will be useful in assessing the role of GH in growth retardation.

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Abstracts from the Literature

Leptin-Replacement Therapy for Lipodystrophy

Severe lipodystrophy is known to be associated with leptin deficiency, insulin resistance, hypertriglyceridemia and hepatic steatosis. Thus, the authors assessed whether leptin-replacement would ameliorate this condition and its complications. Nine female patients (ages 15 to 42 years; 8 with diabetes mellitus) with lipodystrophy of various types, with serum leptin levels of less than 4 mg/ml, and with high insulin levels received recombinant methionlyl human leptin subcutaneously twice a day for four months in escalating dosages (0.03 mg to 0 – 0.4 mg/kg/day) to obtain low, intermediate, and high physiologic serum levels of leptin. During the treatment, the serum leptin levels increased from a mean of 1.3 +/- 0.3 mg per ml to 11.1 +/- 2.5 mg per ml.

The glycosylated hemoglobin values in the diabetic patients decreased, a mean reduction of 1.9%. After four months of therapy, the average triglyceride levels decreased by 60% and the liver volume diminished in size by an average of 28% in all patients. Leptin also led to a discontinuation or a large reduction in the anti-diabetes therapy. The self-reported daily caloric intake also decreased significantly. No major problems or side effects occurred. The authors concluded that leptin replacement improved glycemic control and decreased triglyceride levels in patients with lipodystrophy and leptin deficiency.

Elif AO, et al. *N Engl J Med* 2002;346:570-578.

Editor's Comment: *These investigators demonstrated that leptin deficiency contributes to insulin resistance and other metabolic abnormalities associated with severe lipodystrophy. The reduction of glycosylated hemoglobin associated with leptin therapy is important, reflecting improved diabetic control. This could lead, if the effect persists, to a decrease in the relative risk of retinopathy and/or nephropathy in the diabetic population. The decreased triglyceride levels may reflect a reduced relative risk of adverse cardiovascular events. The alterations that characterize lipodystrophy are known to be refractory to other treatments, and, therefore, this paper reports a novel action of this hormone in addition to its known role in the control of energy homeostasis.*

For those readers wishing more information regarding leptin, consult the article in the last issue (GGH 2002 Vol 18:2), which is entitled "The Endocrine Function of Adipose Tissue" and the article entitled "Molecular Physiology of Leptin and Its Receptor" (GGH 1998 Vol 14:2). Several articles from the literature concerning leptin have been abstracted in GGH since 1998.

Fima Lifshitz, MD

Effect of Growth Hormone Therapy on Height in Children with Idiopathic Short Stature: A Meta-Analysis

The authors reviewed all published (English language) manuscripts and manually searched all issues of the *JAMA*, *Journal of Pediatrics*, *Pediatrics,* and *Acta Paediatrica*, and the meeting abstract books of the *Lawson Wilkins Pediatric Endocrine Society* and the *Endocrine Society* between 1985-2000 for publications (N=761) that reported primary effects of recombinant human growth hormone (rhGH) on the growth of children. From this group, the authors culled those papers reporting adult stature in more than 5 healthy children with "idiopathic" short stature treated with rhGH whose heights were below the 10th percentile at the initiation of treatment and who had "normal" GH secretion (≥10 ng/mL during provocative testing) and in which more than 50% of the starting population completed the study. From this pool, 19 articles describing 10 controlled studies (N=434) and 34 articles reporting 28 uncontrolled studies (N=655) were selected

for more thorough analysis. In both groups the mean age at the beginning of treatment was 10-11 years, baseline growth rates were approximately 4.3 cm/year, and therapy with rhGH was maintained for approximately 5 years.

In the *controlled* studies, adult stature of rhGH-treated children exceeded that of the control group by 0.84 SD (5-6 cm) with the treated group achieving an adult stature of -1.51 SDs and the control group -2.29 SDs. The adult stature of the rhGH-treated group exceeded their pretreatment predicted adult height by 0.54-0.65 SDs (+3.6-4.6 cm). In the *uncontrolled* studies, the adult stature of the rhGH-treated group exceeded their pretreatment predicted adult height by 0.56-0.63 SDs (+3.8-4.5 cm). The authors concluded that administration of rhGH can modestly increase the adult stature of children with idiopathic short stature. They estimated the cost of treatment to be approximately $14,170/cm ($35,000/in). The authors discuss the limitations of this meta-analysis (such as the heterogeneity of the populations treated; absence of data on those children who did not complete the course of treatment with rhGH) and point out that there are no data demonstrating any beneficial effect of treatment on psychological well-being, educational achievement, or vocational advancement.

Finkelstein BS, et al. 2002 Arch *Pediatr Adolesc Med* 156:230-240.

Editor's Comment: *The authors are to be complimented on the completion of an arduous task. Of concern to this reviewer is the inclusion criterion for short stature of height below the 10th percentile. This reviewer cannot imagine that there are any pediatric endocrinologists who prescribe rhGH for otherwise normal children with heights between the 3rd-10th percentiles. One would very much like to see the data reanalyzed to include only children with heights below the 3rd percentile (or -2 SD) at the initiation of therapy. Among the questions that would be of interest to answer are: 1) Was the growth promoting effect of rhGH more apparent in those with the shortest stature? 2) Did the children with familial (intrinsic/genetic) short stature respond more/less favorably than did those with non-familial short stature? 3) Did pre-treatment skeletal maturation influence the linear growth response to rhGH?*

In addition to the data analyzed by Finkelstein et al, two additional reports of the effect of rhGH on adult stature in children with idiopathic short stature have been published. Lopez-Siguero et al[1] observed a mean gain in adult height of 4.5 cm in 30 boys treated with rhGH compared to an historical control group of 42 lads. Wit and Rekers-Mombarg[2] reported that treatment with rhGH (0.17-0.32 mg/kg/week for approximately 7 years) resulted in a gain in adult height SD score of 1.3 versus baseline height in 53 patients with idiopathic short stature (12 born small for gestational age) as compared to a gain of 0.7 SD in an historical control group of 64 subjects. There was an increment of 4 cm in adult height over pretreatment predicted adult height in those children receiving rhGH (+0.8 cm in controls). In children who received the highest dose of rhGH (0.32 mg/kg/week) throughout the study, the increment in adult stature over pretreatment predicted adult height was 7 cm. These authors concluded that higher doses of rhGH led to greater increments in gain in adult height. However, they also concluded that in the absence of proven benefit of greater stature on well-being, the ethical controversy about the administration of rhGH to healthy children, and the high cost of rhGH treatment, "rhGH treatment for (idiopathic short stature) cannot be advised in general." This reviewer would agree with this conclusion.

Allen W. Root, MD

1. Lopez-Siguero JP, et al. *J Pediatr Endocrinol Metab* 2000.13:1595-1602.
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Centers for Disease Control and Prevention 2000 Growth Charts for the US: Improvements to the 1977 National Center for Health Statistics Version

The childhood growth charts used by most centers in North America are the charts produced by the National Center for Health Statistics (NCHS) in 1977. There are a number of problems with those charts that have been overcome in the newly produced charts from CDC. Specifically, the 1977 charts did not fully represent a cross-section of children living in the U.S. They were also deficient in including breast-fed infants. They did not make the transition well, using the recumbent lengths on the infant charts and standing heights on the childrens-adolescents growth charts, and only heights up to 18 years of age were utilized. The new charts follow adolescents up to 20 years of age. The new charts also allow both percentiles and z-scores to be determined and provide body mass index for age charts and smooth the percentile curves.

The national data collection in a series of five surveys between 1963 and 1977 were used to develop the 2000

CDC charts. Other sources of data were also included. There were two important exclusions. Very low birth rate infants were excluded from the infant growth charts and, secondly, all infants excluded from the NHANES III study were also excluded.

The growth charts are not presented here as they are available on the internet (http://www.cdc.gov/growthcharts). They should be very helpful for all physicians and nurses caring for children.

Ogden CL, et al. *Pediatrics* 2002;109:45-60.

Editor's Comment: *We certainly agree that the new growth charts are an improvement over previous charts available for monitoring growth in children in the United States. The editorial on childhood growth charts written in the same journal as an accompaniment to the publication of the growth charts should be carefully read. Careful measurements of children for both height and weight, and the plotting of the data on an appropriate growth chart MUST BE a routine in all pediatric practices.*

Fima Lifshitz, MD
Judith G. Hall, OC, MD

Reduction in the Incidence of Type II Diabetes with Lifestyle Intervention or Metformin

The Diabetes Prevention Research Group, a consortium of 27 clinical centers, conducted a randomized clinical trial involving adults in the U.S. who were at high risk for the development of T2DM. The study was designed to answer three questions: (1) does a lifestyle intervention or treatment with Metformin delay or prevent the onset of diabetes; (2) do the two interventions differ in effectiveness; and (3) does the effectiveness differ according to age, sex, race, or ethnic group. To answer these questions, 3,234 individuals were randomized to one of three treatment groups: (1) standard lifestyle recommendation plus metformin, (850 mg twice daily); (2) standard lifestyle recommendation plus placebo twice daily; or (3) an intensive program of lifestyle modification.

The standard lifestyle recommendation included written information and an annual individual session of 20-30 minutes emphasizing the importance of a healthy lifestyle. The participants in growth 1 and 2 were told to reduce their weight, to increase their physical activity, to follow the Food Pyramid Guide, and to follow a diet the equivalent of a National Cholesterol Diabetes Education Program Step 1. The participants in group 3, the intensive lifestyle intervention group, were to achieve and maintain a weight reduction of at least 7% by following a low fat diet and by performing moderate physical activity such as brisk walking for at least 150 minutes per week. In addition, these subjects participated in a 16-week curriculum promoting dietary education, exercise, and behavior modification.

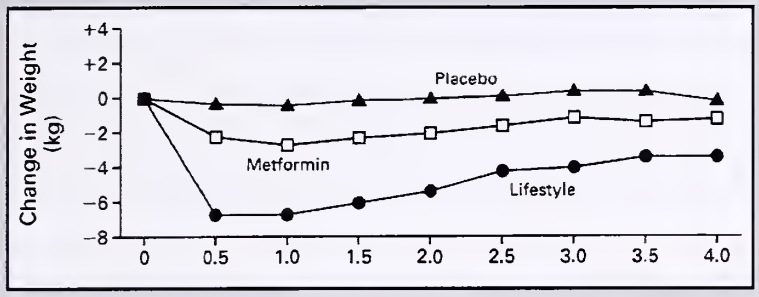
The primary outcome variable was diabetes as diagnosed by an annual oral glucose tolerance test or a semi-annual fasting plasma glucose test. The blinded treatment phase was terminated one year early, because by that time there was evidence of efficacy on the basis of 65% of the planned person-years of observation.

Approximately two-thirds of the subjects in the study were female, 54% were Caucasian, 20% African-American, 16% Hispanic, 5% American-Indian, and 4% Asian. Seventy percent had a positive family history of diabetes. The mean age for the entire group was 50.6, + 10.7 years, the mean weight 94.2 + 20.3 kg; the mean BMI 34 + 6.7, the mean plasma glucose 106.5 + 8.3 mg/dl, and the mean glycated hemoglobin was 5.9%. The mean baseline data were similar in the 3 groups.

In the lifestyle intervention group, 50% achieved the goal of a 7% weight loss by the end of the first 24 weeks and 38% had maintained that weight loss at the last visit. Seventy-five percent participated in 150 minutes of physical activity per week at the end of 24 weeks and 58% maintained that level. Daily caloric intake decreased by a mean of 450 kcal in the lifestyle intervention group, 249 kcal in the placebo group, and 296 kcal in the metformin group. The average fat intake (34.1% of total at baseline) decreased by 6.6 $\pm$ 0.2% in the lifestyle intervention group and by 0.8 $\pm$ 0.2% in the placebo and metformin groups. Participants in the lifestyle intervention group had a much greater weight loss and greater increase in physical activity, than did the subjects in the other groups. The average weight loss was 5.6 kg in the lifestyle intervention group, and 2.1 kg and 0.1 kg in group 2 and 1. (Figure 1)

The incidence of diabetes was 4.8, 7.8, and 11.0 cases/hundred patient years for groups 3, 2, and 1 respectively. The incidence of diabetes was 58% lower in the lifestyle intervention group (group 3) than in the placebo group (group 2) and 31% lower in the metformin group than in the placebo group. (Figure 2) These results were statistically significant and the estimated cumulative incidence of diabetes at 3 years was 28.9%, 21.7%, and 14.4% in groups one, two, and three, respectively. Unfortunately, the study had inadequate power to access the significance of the effects within ethnic groups, but effects did not differ significantly according to sex, race, or ethnic group.

Figure 1



Changes in body weight according to study group. Each data point represents the mean value for all participants examined at that time. The number of participants decreased over time because of the variable length of time that persons were in the study. For example, data on weight were available for 3085 persons at 0.5 year, 3064 at 1 year, 2887 at 2 years, and 1510 at 3 years. Changes in weight and leisure physical activity over time differed significantly among the treatment groups (P<0.001 for each comparison).

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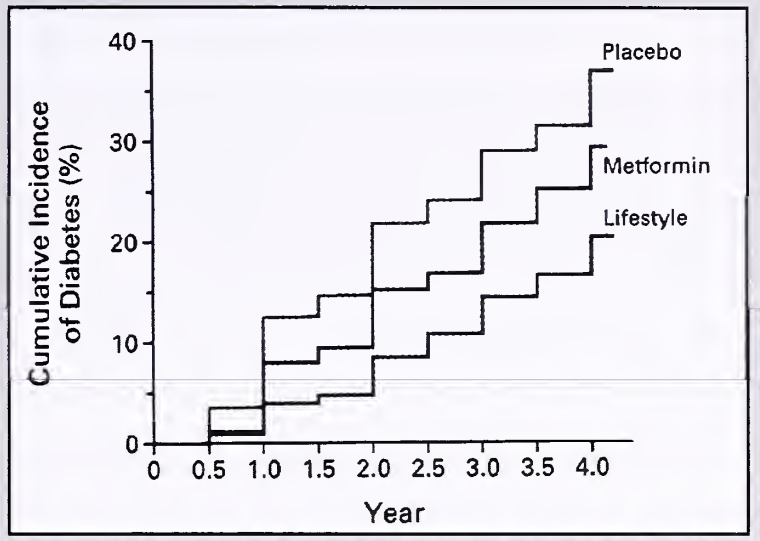
Figure 2



Cumulative incidence of diabetes according to study group. The diagnosis of diabetes was based on the criteria of the American Diabetes Association. The incidence of diabetes differed significantly among the three groups (P< 0.001 for each comparison).

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The authors state the hypothesis that Type II diabetes can be prevented or delayed in persons at high risk for diabetes was proven, and the effects were similar in men and women and in all racial and ethnic groups, regardless of age. The authors point out that their results show a risk reduction associated with lifestyle intervention that is similar to a previous test study conducted in Finland. The current study however, was not designed to test the relative contribution of dietary changes, increase in physical activity and/or weight loss. This is the first study, however, to demonstrate the efficacy of drug therapy in reducing the risk of developing Type II diabetes in high risk individuals.

Diabetes Prevention Group *N Engl Med* 2002;346:393-403.

***Editor's Comment**: This is an exceedingly important publication, as was another significant paper published last year in the New England Journal of Medicine on the prevention of Type II diabetes mellitus by making alterations in lifestyle among subjects with impaired glucose tolerance (N Engl J Med 2001;344:1343-1350). The current study conducted in an older group of subjects has similar implications for children at high risk of developing Type II diabetes. In addition, the current study suggests that metformin, at a relatively modest dose (850 mg bid), can reduce the risk by 31%.*

Most pediatric endocrinologists are faced with increasing numbers of overweight children coming to their clinics for evaluation. Many of these children are at very high risk for the development of Type II diabetes. The clinical armamentarium remains limited. Clearly, studies are needed to confirm the effectiveness of metformin in preventing the onset of Type II diabetes in the pediatric age group. However, previous experiences amongst pharmaceutical companies attempting to recruit and retain children with Type II diabetes for clinical trials suggest that this will be a very difficult task. Such a clinical trial may require nearly as much effort as the clinical treatment of Type II diabetes. Although, most physicians would recommend a change in lifestyle modification for overweight children, the execution of changes in dietary intake and physical activity within the context of a family with varying degrees of motivation remains extremely difficult.

William L. Clarke, MD

***Second Editor's Comment**: This editor must conclude that we may succeed in changing the lifestyle of some obese adults but only in a few obese children, but we should keep trying. With children and adolescents, gentle persuasion will be more effective than parental demand.*

Robert M. Blizzard, MD

Hypospadias and Early Gestation Growth Restriction in Infants

Reports from Europe and the United States have indicated that there is an increasing incidence of hypospadias. This study by Hussain et al involved two tertiary care neonatal intensive care units in Connecticut. It was a retrospective study of 14 years of admissions. It showed a 10-fold increase in hypospadias over the 14 years, from 0.4% of admissions in 1987 to 4% in the year 2000. The increased occurrence of hypospadias among premature infants was associated with intrauterine growth retardation. An increased frequency of hypospadias was also noted among the infants born in the lower percentiles (3rd to 25th).

An association of hypospadias with the smaller quartiles of head circumference (3rd to 25th) was also present. The frequency was highest in first-born infants and those born to older mothers. No association was noted with race, maternal diabetes, hypertension, or pre-eclampsia. No specific teratogens were identified. There does not seem to be an increase of a particular recognizable syndrome in spite of the association with intrauterine growth restriction. The consistent involvement of all growth parameters, i.e., weight, length, and head circumference suggested that hypospadius is related to overall poor intrauterine growth.

Hussain N et al. Pediatrics 2002;109:473-478.

Editor's Comment: *A specific etiology for the observed increase in hypospadias does not seem to be forthcoming. These are obviously real concerns with such a striking change over the last decade. The question of endocrine disrupters and the association of advancing maternal age are important, but no real clarity exists as to their real role at this time.*

Judith G. Hall, OC, MD

Growth, Developmental Milestones, and Health Problems in the First Two Years in Very Preterm Infants Compared with Term Infants: A Population Based Study

Bucher et al report the results of a questionnaire sent to parents of Swiss infants born before 32 weeks of completed gestation. The parents were asked to answer questions concerning weight, body length, head circumference at 24 months of age, developmental milestones, eye and ear problems, long-term medications, fever, cough, and infectious diseases during the last 12 months. Information regarding developmental milestones is recorded in the Swiss Health Carnet given to each parent of a newborn infant. A comparison group for this study included two control infants for each index infant. The second was contacted if the first did not respond. Infants of multiple births or with severe malformations or syndromes were excluded. The control infants had to have been born in the same hospital, at term (after 37 weeks), and within 14 days of the expected date of birth of the index infant, and of the same gender as the index infant.

Three hundred nine infants born between January 1, 1996 and December 31, 1996 were included. Index infants had significantly lower body weight, body length, and smaller head circumference at 24 months *corrected* age as compared to their matched control. The mean weight difference at the age of 2 years (*corrected* for the very preterm infants) was 1.2 kg for boys, and 1.2 kg for girls. The mean difference in body length was 3.5 cm for girls and 3.3 cm for boys. Thirty-three percent of index infants were below the third percentile for length at 24 months *corrected*. The difference in head circumference was small (0.7 cm), but statistically significant ($p < 0.001$). Height and weight parameters were similar in the parents of pre-term and term infants, and in agreement with normal growth standards for adults. In the very preterm infants, there was significant motor delay, increase in eye problems and in use of long-term medications, but no difference in infectious diseases during the prior 12 months. Sitting was not delayed, but walking (mean of 14.5 months vs 13.5 months in controls ($p=0.4$) and drinking out of a cup (50% of each group at 16.5 vs 13.5 months; $p=<0.001$) were delayed. Of the very preterm infants, 16% were unable to walk independent at 18 months *corrected* age. These infants are at increased risk for developing cerebral palsy. The authors state that such a retrospective study can include much bias, but that has been accounted for by utilizing a significantly large control group. The cause of significant growth delay remains unclear. Suggested causes include: (1) decreased length of gestation; (2) insufficient supply of nutrients over prolonged periods of time after birth; or (3) intercurrent illnesses in the first year, such as chronic lung disease which may increase energy requirements and interfere with nutrient intake.

Bucher HU, et al. *Eur J Pediatr* 2002;161:151-156.

Editor's Comment: *The authors recall several studies in which catch-up growth in pre-term infants has been stated to occur up until adolescence, and note that the patients in this study should be followed at least through school age. The data are intriguing however, for several other reasons. First, it is possible that these very young children (less than 30 weeks gestation) may respond with accelerated growth to recombinant growth hormone therapy in much the same way as do children with intrauterine growth retardation. Initiation of such therapy at a young age might significantly improve not only final height, but developmental milestones as well. The discrepancy in head circumference in the very pre-term infant, although minimal, is nonetheless of considerable concern. Thus as the authors point out, it would be important to carefully record growth patterns, and developmental milestones over time in the attempt to define those children who might benefit most from earlier hormonal investigation and intervention. It would appear that the Swiss Minimal Neonatal Data Set is an excellent resource for the collection and analysis on such data.*

William L. Clarke, MD

Adult Height in Advanced Puberty with or without Gonadotropin Hormone Releasing Hormone Analog Treatment

The authors define "advanced puberty" as "the onset of puberty in girls between 8 and 10 years and in boys between 9 and 11 years." (Others might also use the term "early puberty" for such subjects.) In a retrospective assessment of the effect of a gonadotropin releasing hormone agonist (GnRHa - D-Trp^6-GnRH) upon adult stature in children with "advanced puberty," the authors administered GnRHa for 2-2.4 years to 9 adolescent girls with serum estradiol concentrations in excess of 20 pg/mL, and 8 pubertal boys with testosterone values greater than 100 ng/dL who had a pubertal gonadotropin secretory response to GnRH. Mean adult height of treated subjects was compared to that of a control group of untreated subjects. In treated girls, mean adult stature (155.3 cm) was insignificantly different from pretreatment predicted height (151.9 cm). In control females (N=31), mean adult and predicted heights were also similar (157 cm and 156.7 cm, respectively). In both groups, adult heights were close to their target heights. In treated boys, mean adult height (164.1 cm) was less than mean predicted height (173.2 cm) and mean target height (170.4 cm). In untreated boys (N=9), adult height, predicted, and target heights were similar (169.1, 170.8, and 170.2 cm, respectively). The authors concluded: "These data suggest that advanced puberty decreases the growth potential by about 5 cm, and that GnRHa treatment does not prevent this."

Couto-Silva AC, et al. *J Pediatr Endocrinol Metab* 2002;15:297-305.

Editor's Comment: *Luckily, GnRHa did not increase adult stature in girls with "advanced puberty" and may even have led to decreased stature in boys. While under specific and individual circumstances (such as major behavioral problems, disabling physical handicaps, or significant developmental delay), one might consider interruption of pubertal development in subjects of normal adolescent age, to do so for the purpose of achieving a greater adult stature is an unjustified use of agents such as GnRHa. Similarly, the use of recombinant human growth hormone (rhGH) to increase to a minimal extent adult stature in normal but short children is unjustified medically, psychosocially, or financially.[1] Unfortunately, we may shortly expect to read a manuscript in which both GnRHa and rhGH have been administered to children with "advanced puberty."[2,3] At what point did the pediatric endocrinologist cease being a physician-scientist and become a physician-cosmetologist?*

Allen W. Root, MD

References

1. Finkelstein BS, et al. *Arch Pediatr Adolesc Med* 2002;156:230-240.
2. Kamp GA, et al. *J Clin Endocrinol Metab* 2001;86:2969-2975.
3. Kaplowitz PB. *J Clin Endocrinol Metab* 2001;86:2965-2968.

GH Anabolic Effects of GC-Dependent Children with IBD

This pilot study utilizing 6 boys and 4 girls was designed to determine whether rhGH could overcome some of the catabolic effects of chronic glucocorticoid (CG) treatment (24 months) of IBD. Subcutaneous rhGH (0.05 mg/kg/d) was given for a minimum of 6 months. Seven patients continued for 12 months. Body composition changed favorably with increased fat free mass and decreased fat mass. Linear growth velocity increased from 3.5 ± 0.4 cm/yr pre-rhGH to 7.7 ± 0.9 cm/yr after 6 months. The GV persisted for the next 6 months in all 7 treated. Bone calcium accretion increased as did alkaline phosphate specific for bone [(a measure of bone

formation) p = .03]. Fasting and 2 hour post prandial glucose levels, fasting insulin levels, and HbAIC remained in the normal range. The authors concluded that treatment with rhGH at the doses used has beneficial effects in prednisone-dependent growing children, on body composition without detrimental effects in carbohydrate metabolism or the intermediate metabolism of substrates. Larger studies will be needed to assess long term safety and efficacy.

Mauras N, et al. *Metabolism* 2002;51:127-135.

***Editor's Comment**: This well designed study provides encouraging data that rhGH can overcome the anti-anabolic effects of prednisone, enhance the growth rate, and do so without measurable toxicity over 6-12 months. Of particular interest was the disappointing observation that there was no change in the disease activity as determined by the Crohn's Disease Activity Scale adapted for pediatric subjects. There were significant increases in serum levels of IGF-1 and IGF.BP3. The authors suggest that a state of "functional" GH deficiency caused by chronic steroids may be overcome with rhGH administration. It is important to remember that rhGH has not been effective in treating patients with IBD who are not on glucocorticoid treatment. Also of importance is to recall the reports of Rivkees et al and Allen et al who reported the acceleration of growth in glucocorticoid treated children with significant growth retardation who were treated with rhGH. Allen et al reviewed the data of the Genentech National Growth Study in which 83 children with extreme glucocorticoid induced short stature were treated for at least 12 months with rhGH. The authors concluded: (1) growth suppressing effects of chronic GC are counter-balanced by GH therapy; the mean response being a doubling of baseline growth rate, (2) responsiveness to GH is negatively correlated with GC doses, and (3) glycolysated hemoglobin levels increased slightly, but glucose and insulin levels were not altered by GH therapy. These authors summarized: "In a cohort of 83 poorly growing GC-dependent children, we suggest that the growth suppressing effects of GC can be variably overcome by GH. The short term risks of combined GH and GC treatment appear low; potential long term effects require further surveillance and study. Treatment of GC-dependent children with GH remains experimental; children considered for such treatment should be enrolled in studies that facilitate careful monitoring and data analysis." Dr. Mauras and her co-investigators have heeded the suggestion and extended the data. Rivkees et al, Allen et al, and Mauras et al are to be commended for clinical investigation that significantly enhances patient care.*

Rivkees SA, et al. *J Pediatr* 1994;125:322-325.
Allen DB, et al. *J Clin Endocrinol Metab* 1998;83:2824-29.

Robert M. Blizzard, MD

Inadequate Leptin Level Negatively Affects Body Fat Loss During a Weight Reduction Program for Childhood Obesity

These authors report findings of body fat loss in 37 female and 45 male overweight children, ages 10.9 ± 3.5 years, during a weight reduction program and correlated the weight loss with plasma leptin levels. The authors note that a large proportion (40-80%) of the variance in BMI can be ascribed to genetic factors; leptin appears to signal adiposity and leptin levels have not been shown to be predictive of successful weight loss. Leptin levels, although found to correlate positively with indices of general obesity, have not been found to be predictive for the success of weight loss in observational, longitudinal studies of dietary intervention. Some studies have shown that low serum leptin at baseline is associated with greater weight loss. Others have shown, in adolescents, that a greater baseline of leptin concentration correlates with weight reduction.

In the current study, fasting plasma leptin levels were determined and subjects were stratified on their leptin Z-score into low leptin (< –2 SD), high leptin (≥ + 2 SD), or appropriate leptin (≥–2 to ≤ +2 SD), prior to their weight loss. Body fat was determined by BMI and skin fold thicknesses. All subjects participated in a nutritionally balanced meal plan at 60% of the recommended energy allowances for age and sex. Physical activity was monitored, but no attempt was made to alter it. There were no significant differences in physical activity amongst the 3 groups of children stratified by fasting plasma leptin levels. Data was collected at 3 and 6 months which showed that 20 children had high leptin levels, 20 had relatively low leptin levels, and 42 fell in the appropriate leptin level range. There were no statistical differences among the three groups of children at baseline. Mean BMI and skinfold thickness at the end of 6 months were significantly lower than baseline data. BMI reduction was more evident in the subjects with adequate leptin levels but the differences were not statistically significant. Reduction in triceps and subscapular skin folds was also more pronounced in the appropriate leptin production group. The differences in the average of these changes were statistically significant after both 3 and 6 months.

The authors suggest that children with relatively high or low leptin levels are less likely to lose body fat, as determined by skinfold thickness, during a 6 month hypocaloric diet, and that the ability to lose fat may be strictly dependent on genetic and environmental factors. Therefore, when environmental factors are altered, those with hyper or hypo-leptinaemia are less likely to respond to those changes.

Miraglia del Giudice E, et al. *Acta Paediatr* 2002;91:132-135.

***Editor's Comment**: This is an interesting and important manuscript even though some of the data do not reach statistical significance. Researchers have been unable to show that fasting plasma leptin levels are indicators of the probable success or failure of weight-loss programs. Recent data suggest that, in adults, lifestyle changes including weight loss, and increased physical activity can significantly reduce the risk of Type II Diabetes in high-risk adults. The information in groups of patients who might be more amenable to weight loss programs is therefore very important. Further studies are required in order to better understand the etiology of the differences in leptin levels in the 3 groups of children studied by del Giudice. Confirmation of these data would be of great importance.*

William L. Clarke, MD

Preterm Infants Born at Less Than 31 Weeks Gestation have Improved Growth in Cycled Light Compared with Continuous Near Darkness

The neonatal intensive care unit environment cannot possibly replicate the womb for all preterm infants. The purpose of this study was to evaluate the effects of cycled light versus near darkness on health and growth of preterm infants. The study was set up as a randomized interventional study comparing infants receiving cycled light from birth, cycled light at 32 weeks post-conceptional age, and cycled light at 36 weeks of post-conceptional age. Infants receiving cycled light at birth and at 32 weeks post-conceptional age gained weight faster than infants not receiving cycled light until 36 weeks (Fig 1). There was no difference among the groups in length of hospitalization stay, or number of ventilator days, but the power was low for these variables. The authors concluded that cycled light had significant weight gain benefits over near darkness in preterm infants.

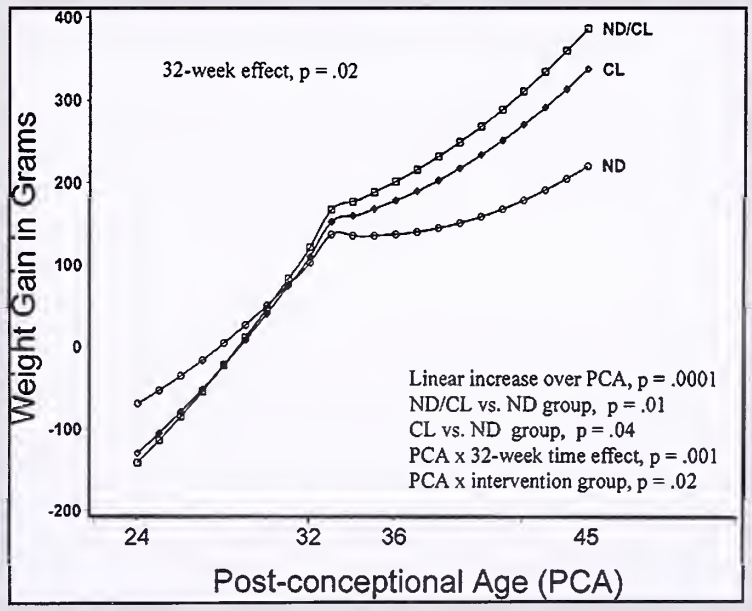
Brandon DH, et al. *J Pediatr* 2002;140:192-199.

***Editor's Comment**: The findings of this study confirm the observations of others who reported that cycled light from birth or beginning at 32 weeks post-conception positively influenced weight gain in preterm infants. The positive effects of weight gain in preterm infants were first reported by Mann et al BMJ 1986;293:1265-7. However, there have been other reports that suggested that continued bright light is detrimental to the health of preterm infants (J Perinat Neonat Nurs 1991;4:47-54 and Infant Behav Dev 1995;18:87-95). Since near-darkness has become the standard of care in nurseries, these findings are important. The presence of significant circadian rhythms provided by maternal cycles even while the fetus is in the intrauterine environment suggest that replicating them after birth may be of benefit. Growth, Genetics and Hormones published an excellent review of circadian rhythms written by Dr. Rivkees in Vol 18, No.1, 2002.*

Cycled light could be important for human development, in addition to the demonstrated benefits in growth. The effects on weight gain, though significant, might only be one part of the benefit of cycled stimulation mimicking intrauterine life for the preterm infant. Potentially, cycled light may also have a major impact on retinal development and other functions.

Fima Lifshitz, MD

Figure 1



Weight gain over time segmented before and after 32-weeks PCA.

Reprinted with permission from Brandon DH, et al. *J Pediatr* 2002;140:192-199.

Letter to the Editor

Dr. Blizzard & Members of the Editorial Board:

I am writing to you because I continue to be disturbed by the fact that many pediatric endocrinologists, including several leaders in the field, continue to ignore published papers casting serious doubts on the validity of the somatomedin hypothesis. The more recent publications of Salmon (whose experiments with Dr. Daughaday half a century ago led to the origins of the hypothesis) have essentially refuted the findings of those original publications, but many pediatric endocrinologists seem to have decided that they do not exist.

I enclose a brief article that I recently wrote summarizing the evidence against the hypothesis: the recent experiments of Salmon and Burkhalter, the experiments done by Derek LeRoith's group at the NIH showing that deletion of the hepatic gene for IGF-I did not impair growth in mice despite a 75% reduction in circulating concentrations of IGF-I; the demonstration that virtually all tissues have growth hormone receptors and do not depend on a circulating messenger to mediate its actions; and the fact that somatomedin is an insulin-like growth factor despite the fact that growth hormone is a counter-regulatory factor that opposes the actions of insulin.

Writings and oral presentations by prominent pediatric endocrinologists continue to cite as gospel the original Salmon and Daughaday papers as though they are unaware of the refutation of those experiments by Salmon and Burkhalter even though they have appeared in peer reviewed journals.

Perhaps *Growth, Genetics & Hormones*, one of the most respected pediatric endocrine publications, might be able to do something about calling the attention of those in the field who need to reexamine the validity of the hypothesis.

Solomon A. Kaplan, MD

Letter from the Editor

The Editorial Board is pleased to respond to Dr. Kaplan's letter of March 27, 2002. His article in *The Endocrinologist* has been updated and is published in response to his letter to the Editorial Board. We believe Dr. Kaplan has succinctly summarized the current knowledge in respect to the inter-relationships between IGF-I, hGH, and growth. Thank you, Dr. Kaplan.

Robert M. Blizzard, MD
Editor-in-Chief

See article on page 38, *Somatomedin Hypothesis: Time for Reexamination.*

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THE CURRENT FRONTIERS OF IN VITRO FERTILIZATION

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INTRODUCTION

In the early 1980s when *in vitro* fertilization (IVF) became a clinical reality it was considered therapy for diseased fallopian tubes. However, its effectiveness soon made it applicable to other causes of infertility, such as endometriosis unresponsive to other therapy, oligospermia with at least a million sperm identified in the ejaculate, and in other possible indications such as infertility of unidentified etiology, and infertility thought to be due to immunological factors.

Improvements in both clinical and laboratory technology at the turn of the millennium made IVF the treatment of choice for all forms of tubal disease (except perhaps iatrogenic sterilization), for endometriosis if infertility was the principal complaint, and for oligospermia regardless of the sperm count, and even for cases of azoospermia in which sperm could be obtained directly from the testis and intracytoplasmic sperm injection (ICSI) used for a single sperm to cause fertilization and pregnancy. It should be said up front, that it appears as if the majority of cases of oligozoospermia are due to genetic causes with the gene primarily carried on the Y chromosome. Therefore, with the use of ICSI, there is a greater transmission of genetic disorders to the next generation since the Y sperm fertilizes the egg. In spite of this, few patients reject this therapy. Occasionally, IVF therapy is used in infertility of undetermined origin and in less frequent conditions, such as the female whose mucous destroys sperm before they can ascend into the uterus.

While the above are the best possible therapeutic options, in current practice, many patients do not receive contemporary therapy. There are numerous reasons for this, but primary among them is that when IVF came into use, the health insurance industry declined coverage on the basis that it was "experimental therapy".

Although IVF is the best possible therapy for several causes of infertility, the insurers continue to deny coverage, resulting in the application of obsolescent therapy for countless patients. For example, diseased fallopian tubes which prevent pregnancy are often surgically repaired because it is covered by insurance. There is reason to believe that contemporary therapy, i.e. IVF, used when medically indicated would be less costly and less risky than the obsolescent therapy supported by the insurance carriers. While some states now have mandated insurance coverage, this is suboptimal because of the restrictions and fixed prices which are often built into the legislation. On a population basis, the United States is now far behind other countries in utilizing IVF. In a study by Collins,[1] it was shown that many other nations are far more frequent users of IVF than the US (Figure 1).

EXPECTATION OF PREGNANCY

The 1998 official IVF Registry Report published in January 2002[2] showed that in the US there were 58,937 cycles involving IVF with a delivery rate per retrieval of 29.1% or 17,150 deliveries. There were 5,273 fresh donor oocyte cycles with a delivery rate for transfer of 41.2% (2,179 deliveries) and 11,228 frozen embryo transfer procedures with a delivery rate per transfer of 19.3% (2,167 deliveries). These percentages are as expected, as fresh donor procedures unequivocally are more successful than frozen embryo procedures. The Registry data are more than three years out-of-date and

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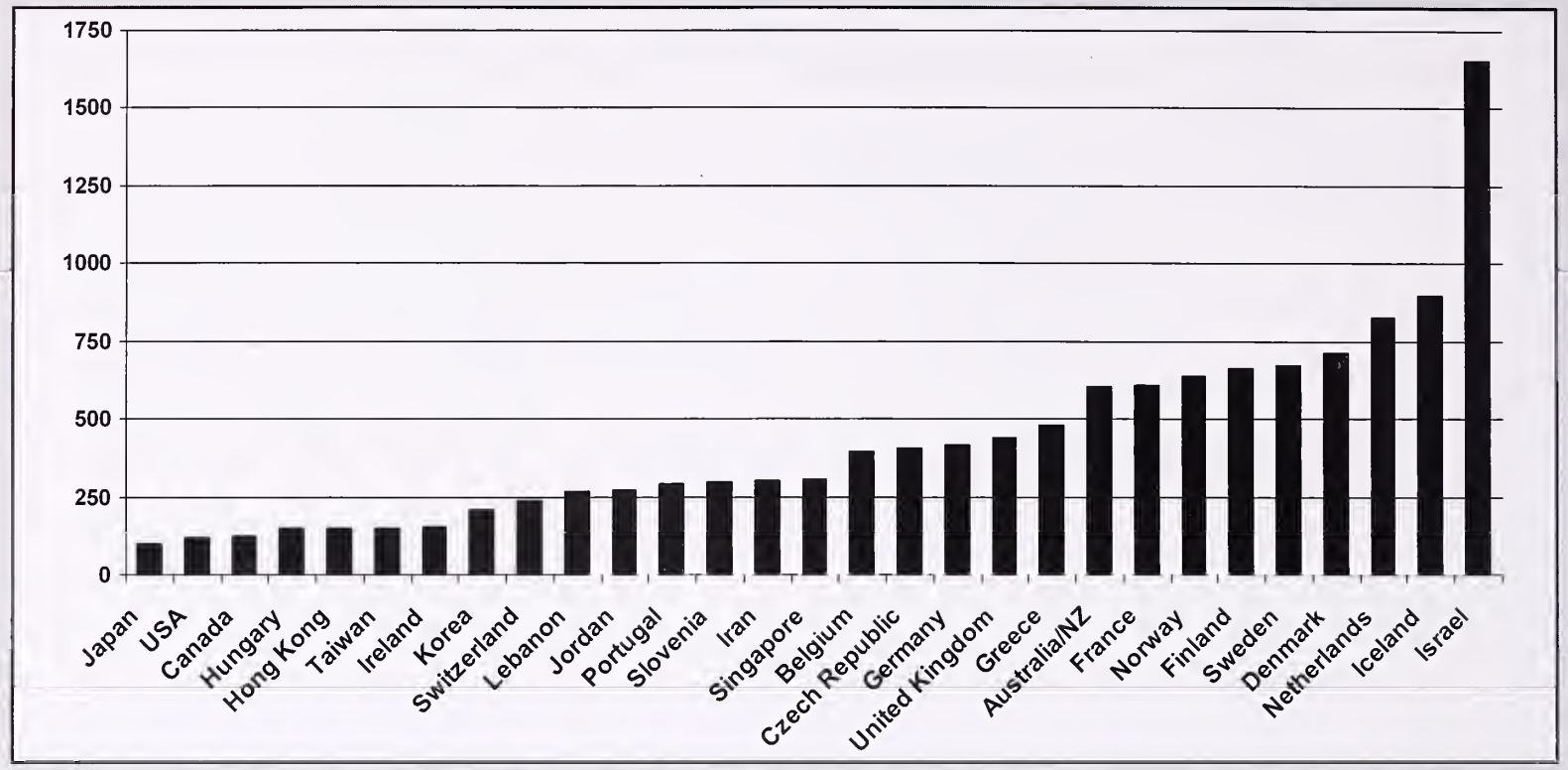
Growth, Genetics & Hormones is supported by an unrestricted educational grant from **Genentech, Inc.**

Figure 1

IVF/ICSI Cycles per Million Population



Adapted from Collins J. Cost-effectiveness of in vitro fertilization. *Seminars in Reprod Med* 2001;279-289.

for a variety of reasons can indeed be misleading to the unwary reader as different assisted reproductive technology (ART) programs have different performance guidelines and different methods of pooling the data.

It has long been known that fecundity, i.e. the probability of pregnancy per month of exposure, declines with the age of the female partner. This age factor cannot be overcome by the use of IVF; thus, therapeutic results reported in the ASRM/SART Registry[2] show a marked age related effect (Table 1). The therapeutic significance is that patients must be further educated about the eroding effect of age on the reproductive process and pregnancy should be undertaken as early as possible.

Multiple pregnancies have been a troublesome problem with IVF. Since the initiation of IVF and of ovulation induction (which also started around 1980) the multiple pregnancy rate in the US as reported by the Bureau of Vital Statistics (Figure 2) has increased each year through 2000, the last date for which data are available. Although the triplet and higher rate decreased slightly in 1999 and 2000, the increase in the rate for twins more than made up for this decrease so that the overall multiple pregnancy rate has increased each year. Examination of the 1998 ASRM/SART Registry reveals that of all deliveries 61.8% were single births, 31.7% of the deliveries were twins, 6.2% were triplets, and 0.3% were quadruplets or more. This is unacceptable and is caused by pressure from both patients and programs alike. They wish to have a high pregnancy rate which can be accomplished with multiple transfers, but at the expense of multiple pregnancies which are undesirable. The goal should be to have a reasonable pregnancy rate with no more than 1% triplets.

Taking all these considerations into account, in 2002 a female who is a good responder, i.e. one who produces at least 5-6 mature oocytes to the required gonadic stimulation, is not over 38 years old, has both ovaries, and has a sperm producing partner, should expect to have a pregnancy 50% of the time with fresh transfer with a risk of less than 1% of having triplets and less than 4% of having twins.

CRYOPRESERVATION

No program in IVF can be considered "full service" unless it offers cryopreservation which can hold frozen excess preembryos for future use. Indeed, in expressing the pregnancy rate for a particular IVF program, a misleading figure is given, unless the pregnancy potential from the frozen material is included. We have published[3] a theoretical model in which a true expression of pregnancy rate resulting from stimulated cycles can be calculated. The interested reader is referred to this publication for full details. Briefly, it is quite impossible to properly evaluate the pregnancy outcome of a particular stimulation cycle unless supplementary pregnancies, if any, from cryopreservation are considered as part of the pregnancy rate of that particular stimulation cycle. This can be done by adding

Table 1

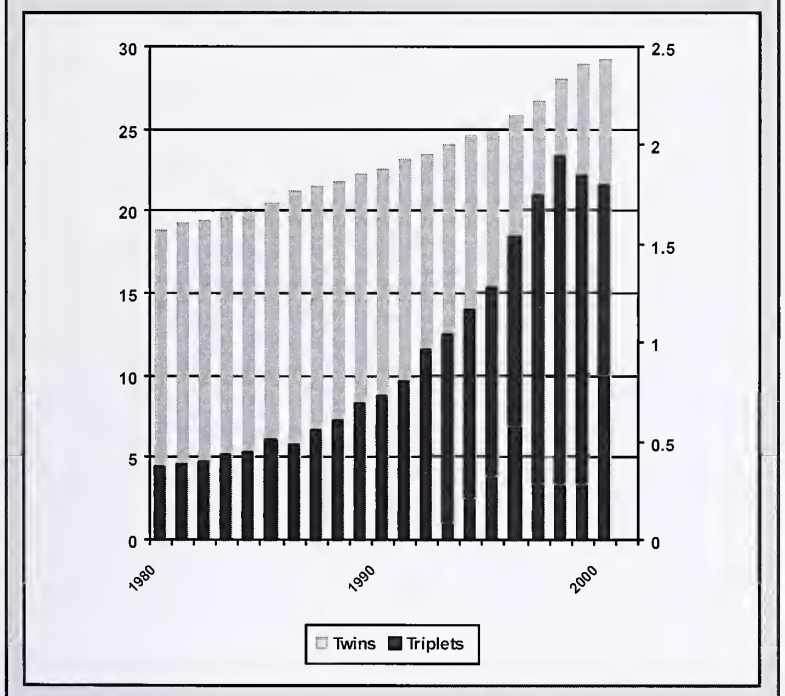
IVF procedures (with and without ICSI) by age group and cause of infertility.

1998 IVF procedures	No. of retrievals	Canceled cycles (%)	Transfers Per retrieval (%)	No. of pregnancies	No. of deliveries	Deliveries Per retrieval (%)	Multiple Births per Delivery (%)
No male factor infertility							
Women <35 years of age	16,648	10.0	93.4	6,878	5,948	35.7	43.4
Women 35-37 years of age	8,524	14.7	94.2	3,109	2,543	29.8	37.9
Women 38-40 years of age	7,063	19.5	92.7	2,006	1,498	21.2	29.0
Women >40 years of age	4,348	24.6	89.9	721	446	10.3	20.2
Male factor infertility							
Women <35 years of age	7,546	7.7	94.7	3,042	2,647	35.1	40.3
Women 35-37 years of age	3,147	11.6	94.8	1,206	1,000	31.8	35.5
Women 38-40 years of age	2,366	14.6	92.9	750	563	23.8	31.8
Women >40 years of age	1,129	19.1	91.9	231	144	12.8	13.9
1998 totals	50,771	13.9	93.6	17,943	14,789	29.1	38.2
1997 totals	44,170	14.0	93.4	15,047	12,302	27.9	39.0

SART/ASRM. ASRM/SART registry: 1998 results. Fertil Steril 2002.

Figure 2

Multiple Pregnancy Rate with IVF and Ovulation Induction



The rating of twins and triplets and more from the Bureau of Vital Statistics, U.S. Public Health Service.

all cryopregnancies to fresh pregnancies, or can be patient specific (ie., considering cryopreservation as augmentation only among patients without a pregnancy from pre-embryos transferred fresh, or from previously transferred frozen material from the same harvest). For the patient-specific concept, cryopregnancies occurring among patients with a previous fresh or frozen pregnancy from the same harvest would be considered additive to the multiple pregnancy rate, i.e. twins, etc., but would be considered as 'delayed' multiple pregnancies. Published results have not reflected the real purpose of cryopreservation; this is shown by the methods of presentation of cryopreservation in the publications of collecting agencies, such as the US Society for Assisted Reproductive Technology, the Great Britain Human Fertilization and Embryology Authority, the Australia-New Zealand Agency, and others. In general these publications report cryopreservation results as unrelated to a particular oocyte harvest or treat a cryopreservation as an additional transfer from the same cohort of prezygotes/pre-embroys, thus diluting the fresh pregnancy rate, as cryoresults are often not as good as fresh results.[3]

Generally speaking, expectation of a pregnancy from cryopreserved material is not as great as from fresh. Although the data are not exactly comparable, the ASRM/SART Registry for 1998 gave an overall pregnancy rate per transfer for fresh oocytes in IVF of 37.8% and 24.3% for cryopreserved material. With careful selection of fertilized eggs prior to cryopreservation, the pregnancy expectation from cryopreserved material approaches that of fresh material.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD has been available since about 1990.[4] By this technique, one or two blastomeres are removed from the preembryos of the 6-10 cell stage and examined for single gene defects by the polymer chain reaction (PCR) or by fluorescent in situ hybridization (FISH) for gross chromosomal defects. Preembryos with defects are discarded and those found to be normal are transferred or frozen for future transfer.

Table 2

PGD referrals (n) according to indication

Chromosomal	647
X-linked	294
Autosomal recessive	290
Autosomal dominant	254
Mitochondrial	6
Two indications	9
Y-chromosome deletion	2
Social sexing	30
Unknown	29

ESHRE PGD Consortium Steering Committee (May 2001) Hum Reprod 17:235, 2002.

Diagnostic ability with PGD is precisely that of amniocentesis which is done at 15-18 weeks of pregnancy or chorionic villus sampling which is done at 10-14 weeks of pregnancy. PGD appeals to those who cannot morally terminate an affected fetus but who do not feel morally bound to implanting an in vitro affected preembryo. It also appeals to those who are prepared to undergo the requirements and expense of PGD and IVF simply to avoid the possibility of an elected termination, even though they may have no moral conflict in aborting an affected fetus.

The opportunity to use PGD is not offered by all centers, but its use is gradually increasing. According to data collected by the ESHRE,[5] in 2001 there were 1,561 PGD procedures reported. The most common cause for referral was concern about chromosomal abnormalities. Specific gene disorders accounted for slightly over one-third of the cases (Table 2). Cystic fibrosis was the most common monogenic disorder.

PGD is not without an occasional error, and its efficiency in relation to fertility factors is somewhat less than IVF because of the limited number of preembryos that can be selected for transfer resulting from the screening out of affected fertilized eggs.

DONOR GAMETES

Donor *sperm* have long been used when infertility was due to sperm deficiencies. Currently, the use of donor *sperm* and *oocytes* can be considered standard practice for those who are prepared to accept nonfamilial genetic material. In some circumstances, donor gametes are used to replace gametes which are likely to or are known to harbor a mutant disease-causing gene. This is particularly valuable when the affected gene is not amenable to preimplantation genetic diagnosis.

When donor *sperm* are used either with or without IVF, the donors are vigorously screened. Requirements differ from center to center. At the Jones Institute the donors must be 18 to 39 years of age, have a semen volume of 2 mL with a sperm count of at least 60 million, with sperm motility greater than 60%, and at least 7% of the sperm must be of normal form by strict criteria. There can be no excess of WBCs. More than 50% of the sperm must survive the cryo-survival test. The family history of the donor must be free of genetic disease. A physical examination must reveal no urethral discharge or genital warts or ulcers. Laboratory screening includes a serological test for syphilis, cytomegalovirus, hepatitis B and C, HIV-1 and HIV-2, and T-cell lymphotrophic virus I and II. Serum tests must be negative for herpes, chlamydia and gonorrhea, and donors must pass a urine test for drug screening. In addition, donors must be free of cystic fibrosis and, if Jewish, tested for Hexosaminidase-A which causes Tay-Sachs disease. Black donors must be free of the sickle-cell trait. Potential Asian or Mediterranean donors with a positive hemoglobin electrophrosesis for thalassemia are eliminated.

Semen quarantine is usually carried out for 6 months at which time the donor is checked for HIV and other possible potential problems before semen is released for use. All this is in accordance with the recommendations of the American Society for Reproductive Medicine (ASRM). Clinical pregnancy rates with donor *sperm*, with or without IVF, are consistent with a normal fecundity rate if there is no impediment to pregnancy on the part of the female.

When donor *eggs* are supplied, the donor has a similar historical review for genetic problems, as well as laboratory studies. However, it is impractical to quarantine an *egg* for six months, as the *eggs* do not freeze nearly as well as the *sperm*. Therefore *egg* quarantine is essentially never done. HIV testing in the *egg* donor is done by the antigen test rather than the antibody test, as a prompt answer can be obtained, although there is some uncertainty as to the time required for the appearance of the antigen. Clinical pregnancy rates for donor *eggs* in IVF are a cut above that obtained by IVF in general - due to the younger age of the donor. The pregnancy rate with donor *eggs* is consistent with the age of the donor and unrelated to the age of the recipient. There is great uncertainty about an upper age limit for the use of donor *eggs*.

ASRM has issued a guideline indicating that donor *eggs* should not be used in a recipient at an age above a woman's normal reproductive life. This guideline probably has been left purposely vague. The guidelines must have been violated as there are accounts of recipient mothers 60 years of age and over. Each program must adopt its own standard in regard to age limit. Some variations in the standard donor egg scenario have occurred. For example, there have been

menopausal grandmothers who were prepared to receive an anonymous donor egg for their daughter - such an egg, of course, fertilized by the daughter's husband. There are no guidelines for these offbeat situations, thus each program must handle them on an individual basis. Calling for assistance might be appropriate, such as the utilization of sociologists, and/or an ethics committee, or other outside resources to establish guidelines and share responsibility for these decisions.

Suffice it to say, when donor eggs are used, and especially if the recipient's age is 40 or above, a preconception medical evaluation is in order. Such an evaluation would look for those conditions which might cause complications during pregnancy or those which might be aggravated by pregnancy, such as obesity, hypertension, and diabetes. Only those women who are totally medically fit should be considered as recipients.

An upper age limit for a perspective father is sometimes an issue *with or without* donor sperm. This seems to arise when a prospective father is 60 or above and marries a much younger wife. One must ask, "Does the program have a responsibility in this circumstance to consider the welfare of the child; specifically, is there any reason to be concerned about how a man of 60, 70 or 80 years of age can function responsibly, mentally and physically, with teenage children?" A program probably has no responsibility here, but the issue is thought provoking.

CONCLUSION AND A FINAL WORD

Prior to IVF it was common for physicians who treated infertility patients to tell them that everything had been tried, and it was now time to consider adoption or a childless future. Basic IVF technology changed much of that, as did the addition of donor gametes for those prepared to accept alien genetic material; the physician is now able to offer an option to essentially all couples. The era of IVF also has made it possible to go beyond the mere solution of the problem of infertility. Preimplantation genetic diagnosis now makes it possible to eliminate disease-causing mutant genes. Thus, we are beginning to diminish the number of children born with handicaps. Such children previously were thought to represent an intrinsic risk of bearing children.

If the era of IVF has written a new chapter in the treatment of infertility, are there additional chapters to be written? To be sure! The aging oocytes represent a challenge. Can they be rejuvenated? I think it will be possible. IVF is inefficient, but changing this represents a problem. With eight fertilized two-cell zygotes in the dish, experience tells us that on average only two or three of these have the potential to progress to a term fetus. We are far from perfect in identifying which ones are the two or three. Can our selection potential be improved? I think it will be possible. Cryopreservation is very efficient for *sperm* but very inefficient for the *egg* due to its size. Can cryopreservation of the egg be achieved? I think it will be possible.

These are only examples. There are several other possibilities - some of which may be considered by some in the realm of science fiction, but all aimed at improving the human condition. Reproductive medicine and its developing technology have placed us in the midst of a reproductive revolution.

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Abstracts from the Literature

Genetic Screening for Maternal Uniparental Disomy of Chromosome 7 in Prenatal and Postnatal Growth Retardation of Unknown Cause

This very enlightening paper from Finland is worth reading by all pediatric subspecialists for its wealth of information. The authors first relate that uniparental disomy (UPD) associated with growth retardation has been found in at least 9 chromosomes (2,6,7,9,14,16,17,20 & 22) and concluded that UPD thus may provide explanations for some cases of growth retardation of unknown cause. Inheritance of *both* parental genomes is essential for normal growth and development.

In their study, these authors focused on UPD of chromosome 7 and particularly on maternal or matUPD7. The study was prompted as matUPD7 has been reported in approximately 10% of patients with Russell Silver syndrome (RSS) and in a few patients with intrauterine growth retardation (IUGR) without RSS.

Basically 2 groups of patients were studied: (1) 39 patients with unequivocal RSS and, (2) 166 patients with unexplained growth retardation but who did not have RSS. The latter group was divided into 2 subgroups: (2a) those with IUGR and postnatal growth retardation (PNGR) and, (2b) those with only PNGR. For final analysis, the RSS patients were separated into 2 subgroups also: (1a) RSS with matUPD7, and (1b) those without mat-7-UPD.

Only 6 of the 205 patients studied had matUPD7and all had RSS. Thirty-three of the 39 in the RSS group did not have UPD. Comparison of these two groups revealed that RSS infants (with or without matUPD7) were significantly shorter at birth than infants in group 2a and 2b. The birth weights and lengths of RSS patients with or without matUPD7 were equally small. However, birth weights did not differ between groups 1a, 1b, and 2a. Notable difference of parental age at birth was observed between group 1a and the other 3 groups. MatUPD7 patients had significantly higher ($p<.05$) maternal age (38 years) and paternal age (40 years) than those in the other 3 groups.

Midparental heights were near average for all groups. Maternal obstetrical complications known to possibly restrict fetal growth (e.g. toxemia, high blood pressure, and alcohol or tobacco use) were reported in 5 (15%) of 33 of group 1b, 24 (26%) of 91 in group 2a, and only in 5 (7%) of the 75 mothers of the PNGR (group 2b).

The authors point out that matUPD7 and growth hormone deficiency (GHD) can occur together as can GHD and other causes of IUGR and PNGR, and emphasize that other metabolic disorders do not exclude matUPD7. MatUPD7 has been reported in 3 patients with cystic fibrosis, all of which were exceedingly short. Consequently the authors advise screening for matUPD7 if abnormally short stature occurs conjointly with cystic fibrosis or other recessive disorders mapped to chromosome 7. However, because matUPD7 is rare among IUGR and PNGR patients, except in RSS, screening will be primarily helpful in this group of RSS patients.

Hannula K, et al. *Pediatrics* 2002:109:441-448.

Editor's Comment: *The long-term natural history of matUPD7 is not yet clear. Fertility and possible transmission of UPD has not been evaluated. For these reasons, and others such as responsiveness to various therapies, screening in appropriate instances is important. All RSS patients should be screened and those RSS patients with and without matUPD7 should be further evaluated to determine the molecular biological differences between the two groups. The authors discuss some possibilities in their manuscript. The entire manuscript is very enlightening and is recommended both for theoretical considerations and factual data.*

Judith G. Hall, OC, MD

Quality of Life and Self-Esteem in Children Treated for Idiopathic Short Stature

This study from Leiden University in the Netherlands dealt with changes in health-related quality of life (HRQOL) and self-esteem in children with idiopathic short stature (ISS) participating in a study on the effects of growth hormone (GH) treatment. There were 36 pre-pubertal children who were randomly assigned to a treatment or to a control group. Children, their parents and their pediatricians completed a HRQOL and a self-esteem questionnaire, 3 times in 2 years. The data indicated that children with ISS did not have lower scores at the start as compared with the normal population, except for social functioning. The pediatricians noticed an improvement in HRQOL in the children in the treatment group. Those in the treatment group did grow significantly more than those in the control group. However, the parents and the children being treated reported no change in HRQOL. Indeed, in some instances they reported being worse than before. The child's satisfaction with height was more related to HRQOL than was measured height. The authors concluded that the assumption that growth hormone treatment improves HRQOL or self-esteem in children with short stature could not be supported by this study.

Theunissen NCM, et al. *J Pediatr* 2002;140:507-515.

First Editor's Comments: *It is widely assumed that short stature may be a handicap and that this condition may result in psychosocial problems, such as ridicule, and mascotism. Indeed, short people might be victims of discrimination and prejudice, often referred to as "heightism". For that reason, many have opted to receive GH with the intent to accelerate growth and improve the final adult height, and in that way improve their psychosocial status. The response to GH treatment in these children appears to be modest, resulting in a possible gain in final height of 5–9 cm, after many years of treatment. However, few studies have approached the concept of HRQOL as an outcome measure of this treatment. In this study, children with a height of more*

than two standard deviations below the mean for age and sex, who were not GH deficient, were found to have appropriate HRQOL and self-esteem, and did not show improvements after GH treatment. The parent's opinion about their social competence after treatment was also not changed. Of interest was the lack of agreement between the informants, who were the patients and parents, with the pediatrician's perception of the effects on quality of life after GH. The relationship between stature, growth, HRQOL and self-esteem might be determined by the expectations of the participants rather than by the improvements in growth. These children, as well as their parents, might have had unrealistic expectations and, therefore, not be satisfied with the treatment, despite improved standard deviation scores for height. Therefore, when we undertake treatment of a non-growth hormone deficient short child, we should consider aspects other than height. GH treatment should not be initiated just because the child is short. An interesting editorial accompanied this article and was written by Basil J. Zitelli in the same issue of the journal, and the reader is encouraged to review that as well. (Journal of Pediatrics 2002;140:493-495).

Fima Lifshitz, MD

children and parents therapy for short stature raises expectations of success. Motivation to be included in GH trials frequently involved the hope of gaining height, yet if expectations were not met through therapy, poor self-esteem and parental anxiety and disappointment were acutely felt by the child. With the variability and unpredictability of results for any particular child, GH therapy becomes an intervention that may be more detrimental than the original complaint of short stature.

Investigators have added another layer of therapy to enhance growth. To delay epiphyseal fusion, gonadotropin releasing hormone agonists have been added to GH treatment regimens. This may potentially compound the iatrogenically introduced fear in the normal short child of being abnormal or affected with a disease that requires 2 medications to treat.

The last issue (GGH 2002 Vol 18:3) has an abstract and commentary regarding the use of LHRHa in advanced puberty. The conclusion of the authors was "these data suggest that advanced puberty (as differentiated from sexual precocity defined as sexual development in girls before the age of 8 years and boys below 9 years) decreases the growth potential by about 5 cm and that GnRHa therapy does not prevent this".

Robert M. Blizzard, MD

Second Editor's Comment: *Dr. Zitelli in his commentary points out with emphasis that offering*

A Gene as a Major Cause of Sotos Syndrome has been Identified

Sotos syndrome is a relatively common neurologic disorder characterized by prenatal and postnatal overgrowth, advanced bone maturation, large skull with acromegalic features, and significant developmental delay. Most cases are sporadic, but autosomal dominant inheritance has been suggested in some instances and autosomal inheritance in a few rare instances. Reports of balanced translocations have pointed to several chromosomal sites as the location of a gene responsible for the syndrome. One of these has led to the identification of mutations of a nuclear hormone receptor cofactor as a major cause of this syndrome.

Kurotaki et al analyzed DNA from a patient with a de novo translocation 46,XX,t(5;8)(q35;q24.1) that had been reported previously by Imaizumi et al. From analysis of a series of overlapping clones, a contig, that covered the break point, they identified a partial sequence that corresponded to a gene originally cloned in mice, *NSD1*. They then isolated and characterized the human *NSD1* showing that it encoded a protein of 2,696 amino acids that is expressed in many tissues including fetal brain, skeletal muscle and kidney, and that the 5q35 breakpoint is located within *NSD1*.

The group next analyzed DNA from 38 patients with the clinical diagnosis of Sotos syndrome. De novo point mutations that would predict truncated gene products with loss of function were identified in four individuals. Fluorescent in situ hybridization (FISH) analysis revealed a common 2.2 Mb deletion in 18 and a smaller deletion in one of 30 patients in whom a suitable chromosomal spread was available. These deletions included the entire *NSD1* gene. In total, a loss of function mutation or a deletion of *NSD1* was found in 77% of patients implicating haploinsufficiency of *NSD1* as a cause of Sotos syndrome.

NSD1 is thought to act as a co-activator or co-repressor of nuclear hormone receptors, such as the androgen receptor, depending on the promoter context of the target gene and the cellular context. In other words, in one cell type *NSD1* may interact with a combination of regulatory factors unique to the cell type to activate a target gene, whereas it may interact with another set of factors to inhibit expression of target genes in another cell type. The mutations thus alter expression of target genes in relevant tissues.

Clinically, the authors state that the identification of a deletion or mutation of this mutated gene on

chromosome 5 will sometimes help in the diagnosis of Sotos syndrome. Investigatively, the knowledge reported in this article will eventually shed light on some of the underlying mechanisms producing human mental retardation and physical growth.

Imaizumi K, et al. *Am J Med Genet* 2002;107:58-60.
Kurotaki N, et al. *Nat Gen* 2002;30:365-366.

***First Editor's Comments**: Sotos syndrome has been considered to be a relatively heterogeneous entity. The identification of the responsible gene(s) will undoubtedly lead to a better definition of the syndrome and a better understanding of the features observed. Sotos syndrome can now be added to the growing list of disorders with microdeletions in which fluorescent probes are available to identify affected individuals.*

In the last few years, identification of individuals with translocations has been instrumental in identifying the genes responsible for many genetic disorders. Sotos syndrome has been considered to be sporadic, even though there were a few reports of parent/child involvement. This discovery clearly confirms that an abnormality in only one allele leads to the syndrome. As in other microdeletions, the size of the deletion may indicate how severely an individual is affected.

Judith G. Hall, OC, MD

***Second Editor's Comment:** The results reported in this paper argue strongly that Sotos syndrome is caused by a partial loss of NSD1 function. The range of nuclear receptors whose action is affected by NSD1 is not known, nor are the target genes whose level of expression are influenced by NSD1. Given the overgrowth features of Sotos syndrome, one would conclude that the relevant genes are involved in controlling growth and maturation, probably at a very basic level. Moreover, one would expect that the mutations lead to loss of co-activation of growth inhibiting genes, loss of repression of growth promoting genes, or some combination of the two. Questions still remain regarding which cell types are involved. NSD1 is known to be expressed in the fetal brain, which presumably explains the CNS manifestations, but the cells responsible for the skeletal features are still not known.*

William A. Horton, MD

β-Cell–Specific Deletion of the IGF-I Receptor Leads to Hyperinsulinemia and Glucose Intolerance but does not Alter β-Cell Mass

Global deficiency of IGF-I receptors result in hypoplasia of pancreatic islet β-cells. In order to examine the role of the IGF-I receptor in an individual tissue, the investigators from the Joslin Clinic and elsewhere developed a mouse model in which there is "knock-out" of the IGF-I receptor on only the pancreatic islet β-cells. All other tissues continue to express the IGF-I receptor normally, and circulating IGF-I concentrations are comparable to values in controls, indicating no generalized absence of IGF-I presence or action. The investigators did so by breeding animals with conditional *Igf1r* targeting by a neomycin selection cassette for exon 3 flanked by *lox*P sites that was subsequently excised with mice expressing *cre* linked to the rat insulin promoter.

β-cell–specific IGF-I receptor "knock-out" mice (KO) survived normally *in utero* and after birth. β-cell mass, insulin, and glucagon content were normal in control and KO animals at 6 months. *In vitro*, islets from KO mice failed to release insulin in response to glucose in a normal manner and basal insulin secretion was not suppressed by IGF-I added to the incubation medium. *In vivo*, fasting glucose levels were similar, but basal insulin and C-peptide concentrations were higher in KO than in control mice. There was impaired glucose tolerance following intraperitoneal glucose. The immediate first phase of insulin secretion was absent, and the second phase was blunted in KO animals while the insulin secretory response to L-arginine was comparable in KO and control mice. KO mice had reduced islet cell expression of the genes encoding important glucose-sensing proteins, including the GLUT-2 glucose transporter, and glucokinase which is the enzyme necessary for glucose phosphorylation. Thus, the β-cell IGF-I receptor is not necessary for β-cell growth, but it is needed for the selective β-cell insulin secretory response to glucose.

Kulkarni RN, et al. *Nature Genet* 2002;31:111-115.

***Editor's Comment:** Present technology has opened the portal to the investigation of the function of cell-specific proteins. One wonders if patients with impaired glucose tolerance, paradoxically increased basal insulin values, and subnormal insulin glucose-specific insulin secretion, present a loss-of-function defect in β-cell IGF-I receptors. This article and the one on page 62 (β-cell Expression. . .) are related and have potential importance in the future treatment of diabetes mellitus.*

Allen Root, MD

Leptin Acts as a Growth Factor on the Chondrocytes of Skeletal Growth Centers

In order to examine the mechanism(s) by which obesity might lead to enhanced linear growth and advanced skeletal maturation relative to chronologic age, these investigators studied the effects of leptin, a 16-k-Da protein product of adipocytes with anorexigenic properties, upon cartilage cell growth and function *in vitro*. They employed mandibular condyles from 6-day-old mice in organ culture for their model of endochondral ossification. Leptin-specific receptors were identified in chondrocytes in the cartilage growth plate; the molecular weight (148 kDa) of these receptors suggested that they were likely to be the intact, biologically active isoform of this class I cytokine receptor. Addition of leptin (0.5 and 1.0 μg/mL) to the organ culture stimulated chondrocyte division in a dose dependent manner, thereby increasing the width of the proliferative zone and the size of the mandibular condyle. Enhanced functional chondrocyte maturation was demonstrated by increased production of chondroitin sulfate and collagen type II after incubation with leptin. The authors also found that leptin increased expression of the IGF-I receptor in chondrocyte precursors and that immunoneutralization of IGF-I prevented the growth and functional effects of leptin, thus suggesting that leptin's actions are mediated by the IGF-I/IGF-I receptor unit. The authors concluded that leptin has direct effects upon cartilage growth and differentiated function.

Maor G, et al. *J Bone Miner Res*;17:1034-1043.

Editor's Comment: *It has been previously reported that leptin stimulates osteoblast differentiation and maturation. However, leptin levels do not correlate with bone mineral density, an index of bone strength that is more closely related to lean body mass than to body fat content or total body weight. Indeed, experimentally central administration of leptin actually reduces bone mass by an as yet unrecognized mechanism. Of concern and consideration in evaluating this study is the need to employ very high concentrations of leptin to demonstrate biological effects, levels far greater than those achieved in vivo even in the most obese subject. Furthermore, there was a biphasic effect of leptin in this system in that, when incubated with 1.5 μg/mL, most of the reported effects were attenuated. Nevertheless, the data are of interest in furthering our understanding of how obesity might mediate its effects on linear growth and cartilage maturation - particularly in the interesting patients who grow despite complete GH deficiency as after neurosurgical removal of a craniopharyngioma or those with septo-optic dysplasia.*

Root AW, Diamond FB Jr. *Pediatric Endocrinology* 2nd ed, Saunders, Philadelphia, 2002, p 65-95.

Allen W. Root, MD

Effect of Supplemental Zinc on the Growth and Serum Zinc Concentrations of Prepubertal Children: A Meta-Analysis of Randomized Controlled Trials

This study performed meta-analyses of all randomized controlled intervention trials that completed the assessment of the effects of zinc supplementation on the serum zinc concentrations and physical growth of pre-pubertal children. A total of 33 acceptable studies with appropriate data were identified by MEDLINE searches and other methods. Weighted mean effect sizes were calculated for changes in height, weight, weight-for-height, and serum zinc concentrations. The authors used random-effects models, extrapolated by meta-regression techniques.

Zinc supplementation produced highly significant, positive responses in height (+0.35 SDS) and weight (+0.39 SDS) increments. Zinc supplementation caused a large increase in the children's serum zinc concentrations (+0.82). Growth responses were greater in children with low initial weight-for-age z scores, and in those aged more than 6 months with low initial height-for-age z scores.

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The authors concluded that interventions to improve the zinc nutriture of children should be considered in populations at risk of zinc deficiency, especially and particularly in those where there are elevated rates of children who are underweight or experience stunting.

Brown KH, et al. *Am J Clin Nutr* 75:1062-1071.

Editor's Comments: *The benefits of zinc supplementation for children's growth have been debated for many years. This meta-analyses conducted by Brown et al showed that zinc supplements probably are of benefit for children in developing countries. It is not surprising that in such populations there are nutrient deficits which can be corrected by specific nutrient supplementation. Underlining the potential nutritional deficiency status of the population studied and reported, there was a higher significant aggregate zinc effect on children's growth in those who exhibited deficits of body weight for height. It might also be inferred that children who do not exhibit growth retardation or body weight-for-height deficits might not be nutrient-deficient, and may, therefore, not benefit from zinc supplementation. It should also be kept in mind that zinc deficiency is difficult to document, and that zinc supplementation, either alone or in combination with other nutrients, is not easily accomplished nor tolerated by children. Zinc supplements are also expensive where they might be needed the most, namely in developing countries. The foods richest in zinc are from animal sources which are also often not accessible in these countries. Children in the United States and other developed countries who ingest a wide variety of meat products are highly unlikely to be zinc deficient.*

I agree with the authors who state in the last paragraph of this article "Because of the important functional consequences of zinc deficiency for children's growth and other health outcomes, interventions to improve zinc nutriture should be considered in those populations at particularly high risk of zinc deficiency. Additional research will be needed to determine whether the mean serum zinc concentration of a population is a useful predictor of response to zinc supplementation. On the other hand, the population mean serum zinc concentration does increase after supplementation, so this measure can be used to indicate whether public health interventions to promote increased zinc intakes are successful." For those interested in this topic, reviewing the original manuscript and its excellent and extensive graphic expression of data will be appreciated.

Fima Lifshitz, MD

Placental-Specific IGF-II is a Major Modulator of Placental and Fetal Growth

A substantial proportion of imprinted genes, i.e., genes expressed from only one parental chromosome, are involved in placental development and fetal growth in mammals. In the mouse for example, *Igf2* is expressed paternally in the placenta and fetus, while its receptor is expressed maternally. Imprinted genes can act directly on the fetus by influencing cellular proliferation and apoptosis; they can also affect fetal growth by influencing placental structure and physiology and the supply of maternal nutrients. Debate over the evolutionary significance of imprinting in mammals has led to the so-called genetic conflict hypothesis or theory of imprinting. It predicts that paternally expressed genes act on the placenta to promote extraction of resources from the mother to enhance fetal growth while maternally expressed genes act to restrain fetal growth to conserve maternal resources for long-term reproductive fitness of the mother. Testing this hypothesis has been difficult because the relevant genes are expressed in both placenta and fetus and their tissue-specific inactivation has not been achieved.

Recently, it has been shown that the mouse *Igf2* has four promoters, one of which, designated P0, directs paternal expression of *Igf2* in the labyrinthine trophoblasts of the placenta. Deleting this promoter through gene targeting enabled Constância and colleagues to study the impact of paternally-directed placental IGF-II on fetal growth. The P0 knockout for *Igf2* was confirmed by in situ hybridization that revealed a marked reduction of *Igf2* expression specifically in the labyrinthine trophoblasts. Expression of *Igf2* from its other promoters was normal in mutant placentas and fetal tissues as were levels of IGF-II in the fetal circulation.

Lack of the P0 *Igf2* transcripts with paternal transmission primarily resulted in placental growth restriction, which was detected early in gestation at embryonic day 12 (E12) of the 19-day mouse gestation. The impaired growth of the mutant placentas remained relatively constant throughout the remainder of the pregnancy (weight of mutant placentas 76%, 82%, 68%, 68% of normal at E12, E14, E16, E18, respectively) suggesting that the paternally-directed, labyrinthine trophoblast-specific *Igf2* transcripts are required to sustain normal growth of the placenta.

In contrast to the early decrease in placenta size, the indirectly affected fetuses became growth restricted only toward the end of gestation. Their weight was 96% of normal at E16, but dropped to about 70% at birth. The ratio of fetal to placental weight increased as

gestation proceeded and was significantly higher for mutant compared to normal pregnancies reflecting the small placenta size.

To address the discrepancy between placental and fetal growth, the authors compared normal and mutant placentas structurally and functionally. Other than size, no obvious differences in tissue organization or cell morphology were detected. They next compared maternal-fetal transport of different radiolabelled compounds, one transferred by passive diffusion and the other by active transport. Their results showed that passive diffusion declines proportionate to the relative reduction in placental size. Active or system A transport, however, increases during mid gestation, apparently compensating for the loss of passive transfer until near the end of gestation when this compensation is insufficient to meet the needs of the fetus and fetal growth drops off. Importantly, the system A transporter has been shown to be a determinant of fetal growth.

In summary, deletion of a placental-specific imprinted transcript results in fetal growth restriction, primarily through a decrease in total nutrient transfer across the placenta. This example of a morphologically normal but small placenta affecting fetal growth supports the genetic conflict theory of imprinting, in which a placental-specific gene expressed from the paternal allele regulates the supply of nutritional resources to the fetus. On the other hand, fetal demand for nutrients is genetically regulated by the level of growth factors such as IGF-I and IGF-II. Increasing fetal size therefore requires a higher level of demand (for example, higher fetal IGF-II) as well as a higher level of supply (by increasing, for example, placental surface area). Reduced fetal size can be the outcome of reduced supply (as in the P0 mutant described here) *or* of reduced demand (for example *Igf1* knockout, which reduces fetal but not placental size). The mouse *Igf2* gene is remarkable in combining the control of both the supply and the genetic demand for maternal nutrients in a single gene.

Constância M, et al. *Nature* 2002;417:945-948.

First Editor's Comment: *This work supports the genetic conflict theory of imprinting showing that placental-specific genes expressed from the paternal allele contribute substantially to the supply of nutrients a fetus receives from its mother. It also shows that the placenta can partially compensate at least for the loss of this paternal effect. It will be interesting to learn more about the nature of the compensation, which represents a potential mechanism to exploit in treating intrauterine growth retardation. It is important to acknowledge, that the relationship between mother and fetus differs substantially between mice and humans, especially with regard to size and duration.*

William A. Horton, MD

Second Editor's Comment: *As a pediatric endocrinologist who has had a special interest in IUGR for many years, I found the reading of the original article most informative. Not mentioned in the abstract or First Editorial comment was the following brief statement, "At birth, P0 mutant pups were 69% of normal birth weight. This was followed by postnatal catch-up growth which was complete by three months of age." While, as Dr. Horton stated above that mice and humans (may) differ substantially, there is a corollary between the catch up growth in these IUGR mice and the catch up growth that is seen in most IUGR human neonates (primarily those without associated dysmorphology) in the first two years of life. Subsequent studies dealing with the genetic conflict theory in humans should be very informative and intriguing.*

Robert M. Blizzard, MD

Insulin-like Growth Factor I and Leptin in Umbilical Cord Plasma and Infant Birth Size at Term

Umbilical cord blood samples were collected from 12,804 consecutive deliveries, and cord plasma samples were collected from 585 singleton infants born in Norway at term after uncomplicated pregnancies. These were analyzed for plasma leptin, IGF-I, IGFBP-1 and IGFBP-3. Data were analyzed following log transformation of IGFBP-1 and leptin values. Linear regression analysis was used to determine the contribution of maternal and infant factors to umbilical levels of these hormones. The mean age of the mothers of these infants was 28 years. Seven percent had smoked at the beginning of the pregnancy, and 36 percent were primiparous. Male infants had a higher birth weight and length than girls, but girls had a higher ponderal index. Leptin and IGF-I levels were higher in the cord blood of female infants than in males. None of the maternal factors which were analyzed, including pre-pregnancy weights, smoking, or number of previous pregnancies were significantly associated with levels of cord leptin. IGF-I, IGFBP-3, and leptin increased proportionately with increasing birth weight. Levels of IGF-I and leptin were the strongest predictors of both birth weight and birth length, and were independent of length of gestation, maternal age, parity, pre-pregnancy weight, smoking and offspring sex.

The authors conclude that their data suggest that the sexual dimorphism in the regulation of leptin and IGF concentrations, which previously was demonstrated in later childhood, may already be established at birth. They also suggest a possible role for leptin and/or the IGF–I system in relation to birth size and to the risk of diseases such as non-insulin dependent diabetes and cardiovascular disease which have been shown to be frequent in low birth weight infants.

Vatten LJ, et al. Pediatrics 109:1131-1135.

Editor's Comment: *These findings have important implications for understanding the relationship between low birth weight and adult morbidity - especially cardiovascular disease, hypertension, and type 2 diabetes. It would appear that leptin, IGF-I, and IGFBP-I, which have been shown to be important factors in growth in utero, may be important in understanding the risk of developing these adult diseases. It would be very important to follow a cohort of children from birth through adulthood with serial measurements of IGF-I, IGFBP-3, and leptin in order to better understand how these factors change over time and how they might contribute to the development of serious adult disorders. Studies such as those by Vatten et al in Norway support the importance of conducting such difficult epidemiological studies.*

William L. Clarke, MD

A Longitudinal Study of the Effects of a Gluten-Free Diet on Glycemic Control and Weight Gain in Subjects With Type 1 Diabetes and Celiac Disease

Amin et al from Oxford reported their findings of longitudinal growth characteristics and glycemic control in children with type 1 diabetes along with celiac disease (CD). Annually, from 1994 and 1998, 230 children with type 1 diabetes were screened starting in the first year after the onset for the presence of IgA and anti-endomysial antibodies (EMA). A total of 10 children were EMA positive and another one was AGA positive, which was 4.8% of the clinic population. Only one patient demonstrated symptoms typical of CD, including failure to thrive and steatorrhea; four complained of some mild abdominal discomfort. Jejunal biopsy showed classical histopathology of CD in all eleven patients. These subjects were matched for age, sex, and diabetes duration with two control diabetic children who were negative for EMA. Height, weight, and HbA_{1c} were measured at the time of diagnosis of CD and every 3 months. Antibody levels were tested every 3 months until negative, and then yearly. The ANOVA model was used to determine the influence of CD on both HbA_{1c} and BMI SDS. The data are presented as mean $\pm$ SEM.

Mean BMI SDS in the CD group was significantly lower (-1.2$\pm$ 0.1 vs. –0.1 $\pm$ 0.1, P=0.005), as was mean weight SDS (-0.7$\pm$ 0.3 vs. 0.5 $\pm$ 0.3, P=0.002) than in those without CD. However, there was no difference between the two groups mean height or C-peptide level. Mean age of diagnosis of CD was 11.2 years (2.2-17.3). The mean duration of diabetes at diagnosis was 3.8 years (0.9-7.2). Mean HbA_{1c} was significantly lower at diagnosis in the children with CD (8.9%$\pm$ 0.3% vs. 9.8% $\pm$ 0.3%, P=0.002), but there was no difference in the mean daily insulin dose in the two groups. The difference in mean BMI SDS between the subjects and the controls was eliminated by 12 months of gluten-free diet (1.1$\pm$ 0.13 vs. 1.0$\pm$ 0.1, P= 0.11). HbA_{1c} levels were lower than in the controls during the period of gluten-free diet (8.3$\pm$ 0.2 vs.10.0$\pm$ 0.2, P=0.002). Insulin requirements increased in both groups, but no difference in those requirements developed between the two groups. Using a general factorial linear model, CD was associated with lower BMI SDS and lower HbA_{1c} across time, independent of other factors such as insulin dose and regime. Also, while on a gluten-free diet, the children with CD had lower HbA_{1c} which was independent of BMI SDS or the insulin dose or regimen. The EMA antibodies tended to disappear while the patients were on the gluten-free diets.

The authors reviewed recent reports regarding the association in children between type 1 diabetes and CD. Prevalence rates range between 1.7 to 10%. However the data on whether intervention with gluten-free diet would be of benefit remain controversial. This is, in part, because there are few longitudinal follow-up data and few age and sex matched controlled studies. The authors note that their findings could have been influenced by the small sample size or the increased input by dieticians which was received by case subjects. They stress, that because the long-term complications of CD include gastrointestinal malignancy, lymphoma, infertility, and osteoporosis, the screening of children with type 1 diabetes at a young age may be cost effective and warranted.

Amin R, et al. *Diabetes Care* 25:1117-1122.

Editor's Comment: *These findings are very intriguing. Many pediatric endocrine clinics are now screening children with type 1 diabetes for EMA or tissue transglutaminase IGA to identify CD. There is controversy as to whether or not children who are*

asymptomatic with their CD will benefit from a gluten-free diet, and whether or not there is any effect of a gluten-free diet on the management of their diabetes. Amin and co-workers have demonstrated that indeed children with CD and type 1 diabetes are anthropometrically different from those children without CD, and that treatment reverses this finding. In addition, there appears to be a treatment benefit on overall glucose control. The authors noted that their data could have been influenced by the frequent visits to the dietician by case subjects. It will be important to determine whether gluten-free diet is of benefit in all children with diabetes, and or whether similar nutritional input to all type 1 diabetic children could improve HbA_{1c} to the extent observed in this study.

William L. Clarke, MD

Risk for Abnormal Outcomes is Increased with Assisted Reproductive Technology

The advent of assisted reproductive technologies (ART) has increased the complexity of care in newborn nurseries. An increased number of premature infants and multiple births are among a variety of risks that occur with the increased frequency of ART. These risks should be shared with all perspective parents (patients).

An article by Schieve et al studied 42,463 infants who were born between 1996 and 1997, and who had been conceived utilizing ART. These infants were compared to the three million plus infants born in the United States during that period. Among singleton births conceived by ART, and born at 37 weeks or after, the risk for low birth weight was 2.6 times that in the general population. The use of ART was also associated with an increased rate of multiple births which also increases the rate of IUGR births and many other complications.

Hansen et al reported on 301 infants conceived by intracytoplasmic sperm injection and 837 infants conceived with in vitro fertilization (IVF). These were compared to naturally conceived infants from the same region. The infants conceived with ART had an increase of birth defects which was greater than double the occurrence among the naturally conceived. The abnormalities involved a broad spectrum of congenital anomalies. The etiology for the increased risk was unclear. However, advanced maternal age, the usual underlying causes of infertility, medications used to induce ovulation and maintain pregnancy, factors associated with procedures such as freezing and thawing of embryos, and delayed fertilization of the oocyte individually or collectively, contributed to this increased risk.

Strömberg et al studied the neurologic sequelae of children born after IVF. Through a population based retrospective cohort assessment, they compared the neurologic outcome of 5,680 children born after IVF against the neurological outcome of 11,360 matched controls. For each of the 2,060 twins born after IVF, a second set of twin controls was used. Children born after IVF demonstrated an odds ratio of 1.7 of needing habilitation services. Among singletons born after IVF, the risk was 1.4. The most common neurologic disorder was cerebral palsy, with a relative risk of 3.7 for all children born after IVF and 2.8 for singletons. Data concerning twins born after IVF was essentially the same as control twins in respect to neurologic sequelae. Twins with low birth rate and prematurity were more likely to require habilitation services. Maternal age did not seem to be a factor in this study.

Multiple births have an increased risk factor for neurologic sequelae and, consequently, Ozturk et al. strongly recommend that no more than two embryos be placed in the uterus while performing IVF.

Hansen, et al. *N Engl J Med* 2002;346:725-730.
Ozturk, et al. *Lancet* 2002;359:232.
Schieve, et al. *N Engl J Med* 2002;346:731-737.
Strömberg, et al. *Lancet* 2002;359:461-465.

First Editor's Comment: *Information regarding the increased risk of problems associated with ART must be shared with the families who are considering using them. Healthcare providers must also be aware of these risks. The increased expenditures associated with ART are not just the cost of the procedure, but also involve the long-term health care costs. Healthcare costs have become more expensive because of these complications, and these are not usually considered when assessing the expenditures of ART.*

Judith G. Hall, OC, MD

Second Editor's Comment: *A dictum of physics is only rarely violated. Specifically every positive force has a negative force and vice versa. Chances are what we take daily. There are no positive assurances about anything except death. Therefore, we should expect that every technology will not be perfect – either in construction of the technology itself, or carrying out of a procedure with the technology and in the results thereof. Thus, we should not be disturbed by some imperfections of the system, although we should continue to try to make it perfect.*

Human error as well as errors of nature also complicate life, including life related to IVF. The Associated Press on July 10th released in newspapers around the world a report entitled "Test Tube Baby Mix-Up Causes Alarm: Birth of Black Babies to White Couple Raises Questions About Reliability of the Program". This occurrence was in England. Such occurrences of error undoubtedly are very rare, but inevitably occur.

Life goes on, but not always without error. The positivities of what IVF has, does, and will accomplish, far outweigh the negativity of the errors of nature and man.

Robert M. Blizzard, MD

Hypovitaminosis D Prevalence and Determinants Among African American and White Women of Reproductive Age: Third National Health and Nutrition Examination Survey, 1988-1994

This study addressed the issue of the prevalence and the determinants of hypovitaminosis D among 1,546 African American and 1,426 white women of reproductive age (15-49). These women were not pregnant and participated in the Third National Health and Nutrition Examination Survey (1988 – 1994). Hypovitaminosis D was defined as serum 25-hydroxyvitamin D concentrations of < 37.5 nmol/L. The prevalence of hypovitaminosis D was 42.4% among African American women as compared to only 4.2% among white women. The presence of hypovitaminosis D was independently associated with low consumption of milk or cereal, less than ideal use of vitamin D supplements, cold seasons, urban residence, low body mass index, and use of oral contraceptives. Even among the 243 African Americans who consumed an adequate intake of vitamin D from supplements (>200 IU/d), 28.2% had hypovitaminosis D. The authors concluded that the high prevalence of hypovitaminosis D among African American women warrants further examination of the vitamin D recommendations for these women. The determinants of hypovitaminosis D among women should be considered when these women are advised regarding dietary intake and supplement use.

Nesby-O'Dell S, et al. *Am J Clin Nutr 2002;76:187-192.*

Editor's Comments: *The report by this group of investigators provided compelling data with irrefutable evidence that vitamin D deficiency constitutes a major unrecognized epidemic in many young black adult women and in 5% of white women of childbearing age. This survey might have shown a much higher prevalence of hypovitaminosis D if it had been performed in the winter. We may also assume that vitamin D deficiency might be equally prevalent among males of the same age and race, although this was not studied. This article clearly documents it is still currently possible to frequently find vitamin D deficiency in the United States, which plagued our ancestors during the 19th century. There are vulnerable populations, such as those who are not exposed to the benefits of sunlight irradiation, and in those who are dark skinned. The latter may not be able to synthesize sufficient vitamin D from the skin to prevent vitamin D deficiency, and may be in need of higher levels of vitamin D intake as compared to their white counterparts. Therefore, the recommendation to examine the dietary recommendations for young black women and men should be quickly undertaken. Since the black population has a high incidence of lactase deficiency and, therefore, not able to tolerate milk, oral vitamin D supplements may be needed.*

In this study there were no measurements of parathyroid hormone levels or the active metabolic vitamin D (25-D hydroxy vitamin D), both of which are very sensitive indicators of calcium homeostasis and vitamin D deficiency. The high prevalence of hypovitaminosis D among "healthy young female adults" is important as vitamin D deficiency is associated with osteomalacia, bone pain, muscle aches, muscle weakness, and fibromyalgia. It also causes secondary hyperparathyroidism, which can precipitate and exacerbate osteoporosis by increasing mobilization of mineral and matrix from the skeleton. Therefore, there is reason for each of us to pay attention to an easily remedied medical problem that affects many of our patients whether they are adults or children.

Fima Lifshitz, MD

β-Cell Expression of IGF-I Leads to Recovery from Type 1 Diabetes

A method by which to reverse the process that leads to destruction of pancreatic islet cells and type 1 diabetes mellitus is the "Holy Grail" that all diabetologists seek. In the present report from Barcelona, the investigators of the School of Veterinary Medicine and Gene Therapy Center succeeded in doing just that in an animal model

in which the key is selective overexpression of IGF-I in β-cells.

Transgenic mice were developed in which mouse IGF-I was linked to the rat insulin promoter and thus targeted to the β-cell, where IGF-I expression was many fold greater than in control animals. In these mice, at 6 months of age there was a 1.5 fold increase in β-cell mass but normal pancreatic insulin content. Circulating concentrations of IGF-I were comparable in control and transgenic animals. The latter did not develop hypoglycemia, hyperinsulinemia, or neoplasms and had normal life span and reproduction.

At two months of age, administration of streptozotocin (STZ) led to the development of insulitis, hyperglycemia, hypoinsulinemia, and death at four months of age in the control groups from two strains of mice (C57BL and CD-1) utilized. In the C57BL mice which overexpressed IGF-I only in the β-cell, STZ lead to transient modest hyperglycemia, impaired insulin secretion, mild but reversible insulitis, and subsequent normal life span. In the CD-1 transgenic mice, hyperglycemia and hypoinsulinemia following STZ were extreme, but again transient with long term survival (Figure). After recovery from hyperglycemia, the growth was normal in the β-cell-targeted IGF-I transgenic animals.

Histological examination in C57BL mice revealed a mild decrease in islet b-cells and budding of insulin containing cells from pancreatic ductal epithelium. Thus, IGF-I appeared to at least partially protect β-cells from destruction while also increasing generation of new b-cell precursors. Since the β-cell IGF-I receptor is found on the β-cell membrane, the high levels of IGF-I synthesized by the b-cell specific IGF-I transgenic mice must be acting in a paracrine or autocrine manner to protect β-cells insulted by STZ.

Histological examination in the CD-1 mice revealed much less severe insulitis in the transgenic STZ treated mice than in the control STZ treated animals. There was slow recovery from insulitis, but with β-cell proliferation and neogenesis, blood sugar and insulin serum levels were restored to normal.

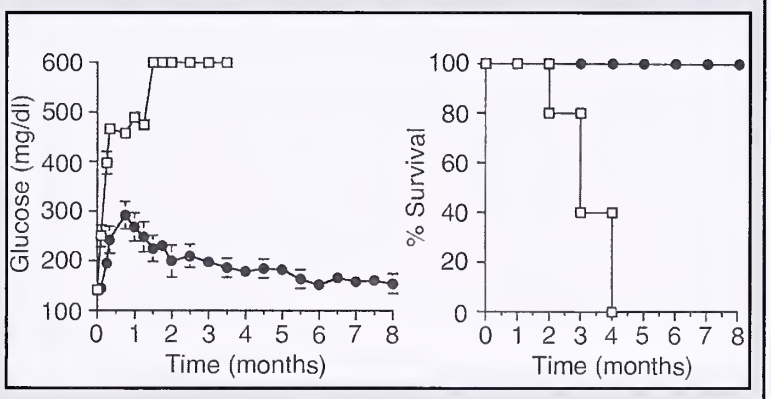
The authors concluded that co-expression of IGF-I and insulin in β-cells protected these cells from permanent destruction by STZ by increasing resistance to the inflammatory insult itself, augmenting β-cell division, and encouraging differentiation of new β-cells. They suggest that IGF-I may be a candidate gene for transfer to pancreatic β-cells in the gene therapy of patients developing type 1 diabetes mellitus.

George M, et al. *J Clin Invest* 2002;109:1153-1163.

Editor's Comment: *This exciting paper raises the possibility that IGF-I might be capable of halting the progression of β-cell loss in patients developing type 1 diabetes mellitus if a method can be found to target this growth factor to the insulted β-cell in the intact patient. Perhaps equally feasible, and possibly even more beneficial, might be the insertion of IGF-I into the β-cells of patients at risk for development of type 1 diabetes mellitus to "protect" or to help them recover from the anticipated insults in the future that will lead to insulinitis. The latter objective may be more useful because the present experiments, which were successful, were conducted in animals that had high IGF-I pancreatic islet contact before the STZ insult. Such an approach would, hopefully, simulate the successful experiment recorded in this article.*

Allen Root, MD

Figure



Blood glucose levels before and after STZ treatment of mice. Glucose concentration was determined as indicated in Methods. Squares, nontransgenic mice (n = 15); circles, transgenic mice (n = 15). Percent survival of nontransgenic (squares; n = 20) and transgenic (circles; n = 20) mice after STZ treatment. Results are mean ± SEM of the indicated mice.

Reprinted with permission from George M, et al. *J Clin Invest* 2002;109:1153-1163.

Growth and Maturation in Marfan Syndrome

The Marfanoid habitus is well known to pediatric clinicians; it is characterized by tall, asthenic habitus. In Marfan Syndrome (MFS), there is multi-organ involvement including eye, heart and muscular/skeletal abnormalities. Erkula et al, largely from Johns Hopkins data, have retrospectively compiled growth pattern data on 180 clinically diagnosed MFS patients. They have generated growth charts and growth velocity charts for infant, children and adolescent males and females. Not unexpectedly, males and females with MFS are larger

at birth, grow at a greater velocity, and end up taller than average. Interestingly, skeletal maturation is also advanced and puberty is earlier when compared to the general population.

These data are extremely important and very helpful for those caring for children with MFS to determine whether a child is outside the expected range for MFS. This and further accumulated data will be very important in respect to the management of the spinal deformities common in MFS, as well as considering either surgical or hormonal therapies to decrease ultimate height.

The study was done using retrospective measurements, primarily from familial cases where the diagnosis had been made on a clinical basis. The authors express some concern about precision of height and weight measurements since they were collected by non-auxologists and because longitudinal data early in life were very limited. Nevertheless, the data are extremely useful in defining the overall natural history of growth in MFS. The authors point out that the excessive linear growth seen in MFS begins prenatally. The growth velocity is consistently higher than that observed in the general population, although body mass does not exceed that in the general population. This combination leads to the slender habitus in MFS.

An important consideration in MFS is the development of idiopathic scoliosis. On average, it develops earlier in children with MFS than in children in the general population. Since it is a common occurrence in MFS, it needs to be screened early and treated aggressively.

The study also documented that skeletal maturation occurs earlier in MFS than in the average population. This is an important consideration when thinking about various therapeutic modalities such as the timing for surgical epiphysiodesis or hormonal therapy to produce cessation of growth and for considering utilizing braces to treat scoliosis.

Erkula G, et al. *Am J Med Genet* 2002:109; 100-115.

***Editor's Comment**: This manuscript should be prime reading for those taking care of MFS patients. Space limits the presentation of the multiple figures presented in the manuscript. These growth charts are available in the original manuscript. These types of growth data are extremely important for relatively rare genetic syndromes and can only be accumulated in centers with enormous experience. Not only is the natural history important to elucidate, but understanding how and when to apply various therapies is extremely important.*

Interestingly, the authors point out that some individuals with MFS are taller than others and, surprisingly, that some MFS patients are obese. Secondary genes or other mutations that affect height and weight are being sought. Such studies may be revealing in better understanding the variations of normal stature as well. It is the careful study of rare genetic disorders that helps to provide better therapy of diseased states and better understanding of normal development. We should be very grateful to this group, which has collected these data over many years. I cannot help but note and be dismayed that it is very difficult to find funding for this type of research and, yet, it is so extremely important. Therefore, we should be even more grateful to the authors and hope that they will be reporting similar data obtained in the studies of other rare genetic growth disorders.

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